REMARKS

Claims 8, 12-13, 15-16, 32, 36-37, 39-41 and 49-53 are pending in this office action. Claims 12 and 36 have been hereinabove canceled without prejudice. Claims 8, 32, and 41 have been hereinabove amended. Claims 8, 13, 15-16, 32, 37, 39-41, and 49-53 are now pending for the Examiner's consideration.

Supports for the amendments are as follows:

Amended Claim	Support
Claim 8	Page 3, lines 26-32 (combination of at least 1 mg to no more than 10 mg)
Claims 32	Page 3, lines 26-32 (combination of at least 1 mg to no more than 10 mg)
Claim 41	Page 20, lines 21-22

No new matter is added.

Applicants request reconsideration of the pending claims in light of the preceding amendments and following remarks.

Claim objection, 35 U.S.C. §112 and 35 U.S.C. §102 (b)

Applicants are grateful to the Examiner that the above claim objection and rejections have been withdrawn.

35 U.S.C. § 103 (a)

Claims 8, 12-13, 15-16, 32, 36-37, 39-41 and 49-53 were rejected under 35 U.S.C. § 103(a) as being obvious over Kania et al. (WO 2001/02369, now US Patent No. 6,531,491) in view of Sweeney et al. Cancer Res. 61 3369-3372, 2001, further in view of Goodman and Gilman, for the reasons set forth on pages 5-7 of the Office Action. Applicants respectfully traverse.

Claims 12 and 36 have been canceled without prejudice, thus the rejections of these claims are rendered moot.

Applicants submit that Kania discloses a broad generic dosage range of "about 0.001 to about 50 **mg/kg body weight**…" (see U.S. Pat. No. 6,531,491, column 21, lines 31-32, *emphasis added*). This broad generic range refers to a class of compounds taught by Kania, not specifically to the specific dosage form of compound of formula 1 (AG013736) of the present claimed. Because the range refers to preclinical animal model, when applied to an average 80 kg human, this broad range translates to 0.08 mg to 4000 mg AG013736 dosage form. The present

claims as amended call for a specific dosage form of 1 to 10 mg AG013736 which are adaptable to human administration as proven by clinical trials.

Applicants submit Rugo et al. (a copy is attached for the Examiner's review) which describes phase I clinical trial results of AG013736. Given strong preclinical evidence for antitumor/antimetastatic activity, a first-in-human phase I trial was conducted to test AG-013736 in patients with advanced solid malignancies. The primary objective was to determine dose-limiting toxicity (DLT) and the maximum-tolerated dose (MTD) of AG-013736. Rugo et al. stated that the initial dosing was chosen based on a modification of the recommendations of DeGeorge et al. (a copy is attached for the Examiner's review), which is one sixth of the body-surface area–adjusted minimal effect dose (MED; 10 mg/kg/d) identified in a 28-day dog study of AG013736. However, this resulted in a calculated safe human starting dose of ***30 mg BID*** (emphases added). Based on the overall safety profile of the first cohort, the average dose evaluated in the first cohort (20 mg BID) was selected as the continuous dose for the second cohort. In subsequent cohorts, escalation of doses by 100% or 40% was planned. However, because of the significant toxicity seen in the first two cohorts, a dose de-escalation schema was used (See Rugo, p.5475, right column, under the heading "Calculation of Starting Dose and Continuous Drug Dosing").

Applicants submit that even at a maximum dose of 30 mg BID as suggested by the DeGeorge calculation based on the animal data, which was even further deescalated based on the teaching of Rugo et al., without hindsight, those skilled in the art will not be able to arbitrarily arrive at 133 times less dosage than the maximum 4000 mg dosage taught by Kania. To arrive at the claimed maximum dose of 10 mg, those skilled in the art would not have been able to arbitrarily further reduce the maximum 4000 mg dosage taught by Kania by 400 times. In contrast, the present inventors have done lengthy clinical human trial studies to arrive at the claimed pharmaceutically acceptable 1 to 10 mg dosage form, as described by the present specification and Rugo et al. below.

Thirty-six patients received AG013736 at doses ranging from 5 to 30 mg by mouth twice daily (see Rugo et al. page 5476, right column, under the heading "Patient Characteristics"). The dose-limiting toxicities observed included hypertension, hemoptysis, and stomatitis and were seen primarily at the higher dose levels. The observed hypertension was manageable with medication. Stomatitis was generally tolerable and managed by dose reduction or drug holidays. The maximum-tolerated dose and recommended phase II dose of AG-013736 is 5 mg, twice daily, administered in the fasted state. No significant drug interaction with antacid was seen. Rugo further teaches on page 5479 that at a starting dose of 10 mg QD/BID, 15 mg BID, 20 mg BID, and 30 mg BID, the Dose Limiting Toxicity (DLT) such as dose limiting hypertension, increased liver function tests, seizure, apnea, hemoptysis, stomatitis, pancreatitis, ischemic

bowel, and thromboembolism were observed (see cohorts 1, 2, and 4 on the table below reproduced for the Examiner's convenience from Rugo et al. page 5480). In contrast, at the claimed dose of 1 to 10 mg, such as 2 mg BID or 5 mg BID, there is none/minimal DLTs (such as dose limiting hypertension, stomatitis, and diarrhea) were observed (see cohorts 3, 5, and 6).

Table 4. Dose-Limiting Toxicities (DLTs)

Cohort (starting dose, No. of patients)	No. of Patients With DLTs	Dose-Limiting Toxicity (No. of patients)
1 (various_fed, 6)	3	Hypertension (3); increased liver function tests (2); seizure (2); apnea (1)
2 (20 mg BID fed, 4)	3	Hypertension (2); hemoptysis (1); stomatitis (1)
3 (5 mg BID fed, 6)	0	None
4 (15 mg QD fed, 6)	3	Hypertension (1); stomatitis (1); pancreatitis, ischemic bowel, and thromboembolism (1)
5 (5 mg BID fasted, 8)	1	Stomatitis (1)
6 (2 mg BID then 5 mg BID fasted, 6)	1	Diarrhea (1)

Abbreviations: BID, twice daily; QD, once daily.
The following doses were administered: 10 mg QD (one patient), 10 mg BID (two patients), 20 mg BID (two patients), and 30 mg BID (two patients).

As taught in the specification as filed, the present inventors have studied dose limiting toxicities (DLT) to arrive at the 5 mg recommended maximum tolerated dose (MTD). For example, on page 20 of the specification, Example 2, Table 4 shows pK results for the AG13736 as a single agent in varying doses for 30 patients with solid tumors on a BID or QD schedule. Cycles were 28 days each. Example 2 also shows a maximum tolerated dose (MTD) of 5 mg BID (see specification, page 20, lines 21-26). The specification further states:" At doses less than or equal to the MTD, the dose limiting toxicities (DLT) was limited to dose 2 stomatitis in 1 patient. Non dose limiting hypertension (HTN) was observed in 7/14 patients and was managed by conventional hypertensive medications." (see specification, page 20, lines 26-28).

The Examiner further contends that "one of ordinary skill in the art would have been motivated to combine the teachings of Kania et al with that of Sweeney et al, because in the Sweeney et al. reference a VEGF (Applicant's compound is known as a VEGF) compound was used with docetaxel, therefore motivating one of ordinary skill to switch the compound of Sweeney et al. with that of Kania et al. and combine with docetaxel because it is well know[n] in the art of cancer that adjuvant therapy are used to give synergistic effect to the cell proliferation." In response, Applicants submit that none of the references teach or even suggest the 1 to 10 mg dosage form of AG 013736 called for in the present claims. Thus the combination claims as written cannot be obvious over the cited references.

Based on the above arguments and claims amendments, it is submitted that the cited references do not teach or suggest the claimed 1 to 10 mg dosage form of AG013736 having the safety profile acceptable for human uses. Applicants respectfully request that the obviousness rejection of claims over Kania in view of Sweeney and Goodman and Gilman be withdrawn.

Double Patenting

Claims 8, 12-13 15-16, 32, 36-37, 39-41 and 49-53 stand rejected under the judicially-created doctrine of obviousness-type double patenting over claims 1-11 of U.S. Patent No. 7,141,581, for the reasons set forth on pages 8-9 of the Office Action. Applicants respectfully traverse.

Claims 12 and 36 have been canceled without prejudice, thus the rejections of these claims are rendered moot.

The remaining claims 8, 13, 15-16, 32, 37, 39-41 and 49-53 have been hereinabove amended to recite still narrower dosage ranges and Applicants submit the following arguments. The claims of the '581 patent are directed to methods of treatment with various compounds and without any specific dosing ranges, much less the specific dosing ranges having acceptable human safety profile. Nothing in the claims of the '581 patent teaches or suggests any dosing range, much less the amended range of 1 to 10 mg, such as 5 mg, nor using the specific range of 1 to 10 mg of the presently recited single compound AG013736. For the same reasons as discussed above, claims 8, 13, 15-16, 32, 37, 39-41 and 49-53 are not rendered obvious by the '581 claims. Moreover, claims 49-53 further recite a still narrower dosing range of 1 to 10 mg, specific types of cancer, and a dosing frequency of twice per day, and in combination with a specifically selected additional anti-cancer agent (docetaxel and gemcitabine, in claims 50 and 51, respectively). Nothing in the '581 patent teaches these specifically claimed methods with sufficient specificity to render the claims obvious. Thus without pure hindsight, those skilled in the art would not have been motivated to arrive at the claimed dosage having acceptable safety profile for human use.

Applicants respectfully request that the double-patenting rejection be reconsidered and withdrawn.

Conclusion

Applicants believe all pending claims are now in condition for allowance. Should there be any issues that have not been addressed to the satisfaction of the Examiner, Applicants invite the Examiner to contact the undersigned attorney.

If any fees other than those submitted herewith are due in connection with this response, including the fee for any required extension of time (for which Applicants hereby petition), please charge such fees to Deposit Account No. 16-1445.

Respectfully submitted,

Date: August 28, 2008

/Elsa Djuardi Lemoine/
Elsa Djuardi Lemoine
Attorney for Applicants
Registration No. 45,963

Pfizer Inc.
Patent Department
10555 Science Center Drive
San Diego, California 92121
Phone: (858) 638-6117
Fax: (858) 678-8233

Cancer Chemother Pharmacol (1998) 41: 173–185

ORIGINAL ARTICLE

Joseph J. DeGeorge · Chang-Ho Ahn
Paul A. Andrews · Margaret E. Brower
Diana W. Giorgio · M. Anwar Goheer
Doo Y. Lee-Ham · W. David McGuinn
Wendelyn Schmidt · C. Joseph Sun
Satish C. Tripathi

Regulatory considerations for preclinical development of anticancer drugs

Received: 19 December 1996 / Accepted: 2 June 1997

Abstract The entry of new anticancer treatments into phase I clinical trials is ordinarily based on relatively modest preclinical data. This report defines the battery of preclinical tests important for assessing safety under an Investigational New Drug application (IND) and outlines a basis for extrapolating starting doses of investigational anticancer drugs in phase I clinical trials from animal toxicity studies. Types of preclinical studies for the support of marketing of a new anticancer drug are also discussed. This report addresses differences and similarities in the preclinical development of cytotoxic drugs (including photosensitizers and targeted delivery products), drugs used chronically (chemopreventive drugs, hormonal drugs, immunomodulators), and drugs intended to enhance the efficacy (MDR-reversing agents and radiation/chemotherapy sensitizers) or diminish the toxicity of currently used anticancer therapies. Factors to consider in the design of preclinical studies of combination therapies, alternative therapies, and adjuvant therapies in the treatment of cancer, and to support changes in clinical formulations or route of administration, are also discussed.

Key words Antineoplastic agents · Toxicity tests · Toxicology · Guidelines · Phase I clinical trials

This article is not an official FDA guidance or policy statement. No official support or endorsement by the Food and Drug Administration is intended or should be inferred

J.J. DeGeorge · C-H. Ahn · P.A. Andrews (✉)
M.E. Brower · D.W. Giorgio · M.A. Goheer ·
D.Y. Lee-Ham · W.D. McGuinn · W. Schmidt ·
C.J. Sun · S.C. Tripathi
Division of Oncology Drug Products,
Center for Drug Evaluation and Research,
Food and Drug Administration, 5600 Fishers Lane HFD-150,
Rockville, Maryland 20857, USA
Tel. (301)-594-5782; Fax: (301)-594-0499;
E.mail: andrewsp@cder.fda.gov

Introduction

Malignant, nonresectable cancers are life-threatening, and aggressive measures are used in treating them. Antineoplastic therapies frequently include toxic chemicals or biological products that are designed to destroy tumor tissue or halt cell replication. Despite the serious toxicities of many anticancer drugs, careful dosing, clinical monitoring and prompt treatment of toxicity makes the side effects less threatening to a patient than their disease. Since it is recognized that doses of anticancer drugs high enough to kill cancer cells usually induce serious side effects in patients, the preclinical testing of oncology drugs differs from testing of nononcology drugs. The Division of Oncology Drug Products within the Center for Drug Evaluation and Research (CDER) at the US Food and Drug Administration (FDA) recognizes the urgency of development of new anticancer drugs and the need to rapidly move promising agents into clinical studies. This report offers a regulatory perspective on the preclinical development of new anticancer drugs that is intended to clarify the differences from the preclinical testing of nononcology drugs and to describe the data that are important to support human testing and eventual marketing.

The types of preclinical studies expected for support of clinical trials and then marketing of a new drug depend on both the intended use of the drug and the population of patients being studied and treated. In situations where potential benefits are greatest (advanced, life-threatening disease), greater risks of treatment toxicity can be accepted and the required preclinical testing can be minimal. In cases where the patient population is free of known disease (e.g. adjuvant therapy or chemoprevention) the acceptable risks are much less and preclinical evaluation should be more extensive [32]. The toxicities of many modulating agents intended to enhance the efficacy or diminish the toxicity of anticancer agents are more similar to those of

nononcology therapies. However, these modulating agents could enhance the toxicity or diminish the activity of cytotoxic drugs by altering their toxicodynamics, pharmacodynamics, and pharmacokinetics. Thus, toxicological evaluation in combination with the modulated cytotoxic drug is an important part of preclinical development.

The following considerations are offered in an effort to balance the risks to be borne by the proposed patient population and the realities of drug testing in humans. The differences in preclinical testing between cytotoxic, chronic (i.e. adjuvant therapy, chemopreventive drugs, hormonal drugs, and immunomodulators), and modulating therapies are emphasized. Issues of chemistry and manufacturing controls, clinical study design, and development of biologic agents for cancer treatment are beyond the scope of this report. If the appropriate preclinical development strategy remains uncertain after contemplating the following considerations, then sponsors are encouraged to initiate pre-IND discussions with Division staff regarding their preclinical study plan.

General considerations for anticancer drug development

Preclinical studies of anticancer agents

The safety of first-time use in humans is assessed through preclinical studies of pharmacodynamics, pharmacokinetics (toxicokinetics), toxicity, and their relationships. The purposes of these safety studies are: (a) to determine a starting dose for clinical trials that is both reasonably safe and allows for possible clinical benefit for the patient, (b) to identify potential end-organ toxicities and determine their reversibility, and (c) to assist in the design of human dosing regimens and escalation schemes for clinical trials. Animal toxicity studies most effectively accomplish these objectives when performed using schedules, durations, formulations, and routes comparable to those proposed in clinical studies. Use of longer duration preclinical studies may lead to underestimates of the appropriate clinical dose, while shorter studies may not identify cumulative dosing toxicities. The toxicity studies should generally conform to the protocols recommended by the National Cancer Institute for toxicology assessment for anticancer agents[1] and are expected to be conducted in accordance with Good Laboratory Practices (GLP) [16, 17]. When studies are not performed according to GLP, deviations should be documented and the potential impact of these deviations on study outcome and credibility should be described [16, 17].

Typically, only two toxicology studies are essential to support initial phase I clinical trials in patients with advanced cancers (Table 1). The first of these is usually a study in rodents that identifies doses that produce life-threatening and non-life-threatening toxicity. The second study should determine whether doses identified as tolerable in rodents produce life-threatening toxicity in a non-rodent species. At least one of these studies should assess clinical signs, body weight, food consumption, clinical pathology, and gross pathology over a range of doses from nontoxic to toxic and should include an examination of histopathology at doses that cause toxicity (or at the highest dose tested). Genotoxicity tests are not generally needed for cancer chemotherapies to support testing in phase I clinical studies unless healthy volunteers will be entered into the study.

While not essential, information on the pharmacodynamics and pharmacokinetics of drugs is extremely valuable for supporting the safety profile and can significantly contribute to the efficiency of drug development. A phase I study may be conducted with no in vitro or in vivo preclinical pharmacodynamic information, but preclinical studies on biological activity and efficacy can substantially aid in clinical study design. Such studies help estimate effective dosages, dosing schedules, and optimal plasma concentrations. This information is likely to be particularly useful when developing noncytotoxic agents. It may be desirable to develop such agents (e.g. immunomodulators) by escalating the human dose to a pharmacodynamically active range rather than to the maximum tolerated dose (MTD). Pharmacokinetic data can be gathered as a part of pharmacology or toxicity studies and do not usually need to be collected separately. Single- and multiple-dose pharmacokinetic studies in the most appropriate species are best performed using dosing schedules, durations, and routes comparable to those that will be used in clinical studies [15]. The pharmacokinetic information obtained assists the evaluation of animal toxicity and efficacy, and may suggest modifications in the intended dose, route or schedule for the clinical trial. The importance of the parameters being measured will vary depending on the clinical trial design and therapeutic classes as discussed in the subsections below. In combination with pharmacodynamic data, this information can be used to help calculate initial doses in humans that have a greater likelihood of activity without adversely affecting safety, and can contribute to optimal dose escalation in early clinical studies.

The proposed therapeutic indication, the outcome of early clinical development, the nature of toxicities seen in animals and in humans, and the projected duration of clinical treatment all determine the preclinical studies necessary to support a New Drug Application (NDA). In general, for oncology drugs, sponsors should conduct toxicity studies using the same schedule and duration of administration as the intended clinical treatment cycle (Tables 1–3). Cytotoxic drugs used to treat advanced disease rarely need studies with more than 28 days of dosing submitted with the NDA (Table 1). In contrast, for drugs intended for continuous

[1] The Developmental Therapeutics Program; Division of Cancer Treatment, Diagnosis, and Centers; National Cancer Institute (Rockville, MD USA) may be contacted for protocol details

Table 1 Preclinical studies for cytotoxic oncology drugs

Stage	Category	Issues to be addressed	Studies considered important[a]	Studies considered useful
IND	All cytotoxics	Starting dose, end-organ toxicities	Rodent[b] and nonrodent[c] toxicology[d]	
		Genetic toxicity	Genetic toxicity panel[e]	
		Effective concentrations, schedule		Pharmacokinetics, pharmacodynamics
	Modifications for Special Categories			
	Photosensitizer	Systemic toxicity	Toxicology studies in subdued light	
		Phototoxicity		In vivo study with illuminated skin
		Plasma $t_{1/2}$		Pharmacokinetics
	Antibody conjugate	Stability	Stability in plasma	
		Toxicity of drug alone	Toxicology in one species	
		Specificity	Human tissue screen	Activity in cell lines ± target antigen
		Pharmacokinetics		Pharmacokinetics
	Liposomal delivery	Drug product toxicity	Include free drug and blank liposomes in toxicity testing	
		Pharmacokinetics versus free drug	Pharmacokinetics	
	Depots	Drug product toxicity	Include free drug and empty depot in toxicity testing	
		Toxicity to contacted tissues	Histopathology of depot site	
NDA	All cytotoxics		Rodent and nonrodent toxicology[a,g], genetic toxicity, stage C-D teratogenicity[f] in rodents and nonrodents	Targeted special toxicity

[a] In general, the schedule and duration of administration in the toxicology study should mimic the clinical trial
[b] Should determine the dose severely toxic to 10% of the animals (STD_{10})
[c] Should determine toxicity of one-tenth the rodent STD_{10} on a mg/m^2 basis
[d] One study should include histopathology
[e] Only for phase I testing in normal volunteers or patients believed to be disease-free
[f] Should be submitted during development
[g] Studies with more than 28 days of dosing are rarely needed

daily administration such as for chemoprevention, adjuvant therapy, or long-term hormonal or immunomodulation therapy, chronic studies should be conducted up to a maximum of 6 months in rodent and 12 months in nonrodent species (Table 2). International Conference on Harmonization (ICH) stage C-D[2] reproductive toxicity studies in a rodent and a non-rodent species are important components of the preclinical evaluation of anticancer drugs and should be submitted early in development [14].

Carcinogenicity studies are not required for cytotoxic drugs used to treat advanced systemic disease, but can be important in the assessment of drugs intended for chronic use for chemoprevention, adjuvant, or hormonal therapy when patients are likely to have a long survival [18]. The current standard is the 2-year rodent bioassay [47], although alternatives may be suitable [20]. Depending upon the nature of toxicities seen with the drug or drug class in animals and in humans, targeted special toxicity studies to support NDA filing may also be needed. For example, in the development of anthracyclines and platinum drugs, which are known to have cardiotoxic and ototoxic potential, respectively, additional preclinical cardiotoxicity and ototoxicity studies have been useful [11, 28, 36, 46]. In addition, neonatal reproductive toxicology and DNA adducting studies have been useful in the development of antiestrogenic agents [5, 30, 31, 37, 44, 45]. A discussion with FDA staff on the preclinical studies needed for marketing approval for a particular drug is recommended at or before the end of phase II clinical studies.

[2] ICH stage A-B, C-D, and C-F reproduction toxicity studies correspond to the previously designated segment I, II, and III studies which are defined by daily administration of drug, respectively, during the period from premating to implantation, implantation to birth (period of organogenesis), and implantation to sexual maturity [14]

Table 2 Preclinical studies for noncytotoxic, chronically administered oncology drugs

Stage	Category	Studies considered important	Studies considered useful
IND	All noncytotoxic chronic therapy	Rodent[a] and nonrodent[b] toxicology[c,d]	Pharmacokinetics, -dynamics
	Modifications for special categories		
	Adjuvant therapy	Genetic toxicity panel[e]	
	Chemopreventive	Toxicology studies should also define NOAEL, Genetic toxicity panel	Efficacy studies Carcinogenicity[e] Stage A-B reproductive toxicity Stage C-D teratogenicity[e]
	Hormonal	28-day toxicology studies usually suffice for limited phase I/II testing in advanced cancer, genetic toxicity panel[e]	
	Immunomodulator	28-day toxicology studies usually suffice for limited phase I/II testing in advanced cancer, genetic toxicity panel[e], define dose versus immunologic response curve to identify shape (bell-shaped?) and surrogate markers	
NDA	All non-cytotoxic chronic therapy	Toxicology studies of equivalent duration to labeled use up to 6 months in rodents and 12 months in nonrodents, genetic toxicity panel, carcinogenicity[f], stage C-D teratogenicity in rodents and non-rodents	
	Additional for hormonal	Stage A-B reproductive toxicity	Stage C-F reproductive toxicity, neonatal reproductive tract toxicity, DNA adducting (drug specific)
	Additional for chemopreventive	Stage A-B and C-F reproductive toxicity carcinogenicity (always)	

[a] Should determine the dose severely toxic to 10% of the animals (STD_{10})
[b] Should determine toxicity of one-tenth the rodent STD_{10} on a mg/m^2 basis
[c] In general, the schedule of administration in the toxicology study should mimic the clinical trial with a duration as long as the intended clinical study up to 6 months in rodents and 12 months in non-rodents
[d] One study should include histopathology
[e] Expected prior to clinical testing in patients with low risk of cancer recurrence, or testing in healthy volunteers
[f] May be unnecessary depending on intended patient population [18]

Starting doses and dose escalation

As described above, one of the primary goals of preclinical studies is to estimate a safe starting dose for the initiation of phase I trials in humans. The starting dose for clinical trials with cytotoxic drugs for oncology indications has traditionally been one-tenth the dose lethal to 10% of rodents on a body surface area basis (milligrams per meter squared) [23, 29, 35]. Studies that actually measure death as an endpoint, however, are not required so long as the dose range studied includes doses that cause severe, life-threatening toxicity. Thus, the starting dose is generally now chosen as one-tenth of the dose that causes severe toxicity (or death) in 10% of the rodents (STD_{10}) on a milligrams per meter squared basis, provided that this starting dose, i.e. one-tenth the STD_{10}, does not cause serious irreversible toxicity in a nonrodent species [29, 35]. If irreversible toxicities are produced at the proposed starting dose in nonrodents (usually dogs) or if the nonrodent is known to be the more appropriate animal model, then the starting dose would generally be one-sixth of the highest dose tested in nonrodents that does not cause severe, irreversible toxicity[3]. In some cases, rodents or dogs may not be appropriate species because they do not model the relevant human biochemical or metabolic processes. For example, folate pools in rodents greatly exceed those in humans [4], so that rodents are generally inappropriate species for testing antifolates. Also, dogs poorly predict the toxicity of some platinum analogues, and an alternate animal model might be preferred [34]. Knowledge of relevant physiological, biochemical, and pharmacokinetic differences between humans and animal models can help determine the most appropriate species to be used for selecting a starting dose. Whenever feasible, these starting doses should be

[3] This calculation is the same as taking one-third of the toxic dose low (TDL) [29, 35]. We believe the current expression of "one-sixth the highest non-severely toxic dose" is simpler and can be applied to the data more universally than taking, in practice, "one-third the dose which causes toxicity but when doubled does not kill the nonrodents". Frequently, the TDL cannot be technically defined in many studies

Table 3 Preclinical studies for modulators of oncology drugs

Stage	Category	Issues to be addressed	Studies considered important[a]	Studies considered useful
IND	All modulators	Starting dose, end-organ toxicities	Rodent[b] and non-rodent[c] toxicology[d]	
		Genetic toxicity	genetic toxicity panel[e]	
		Effective concentrations, schedule		Pharmacokinetics
	Additional studies for special categories			
	MDR modulator	Combination toxicity	One species at minimally and significantly toxic doses of cytotoxic	In vivo efficacy of combination
		Pharmacokinetic perturbations	Pharmacokinetics	
	Chemosensitizer	Combination toxicity	One species at minimally and significantly toxic doses of cytotoxic	
	Radiation sensitizer	Delayed toxicity to normal tissues		Skin/leg contracture
	Chemoprotection	Combination toxicity, tumor protection	In vivo efficacy of combination with histopathology	
NDA	All modulators		Toxicology studies of equivalent duration to labeled use up to 6 months in rodents and 12 months in non-rodents, genetic toxicity, stage C-D teratogenicity in rodents and non-rodents	Targeted special studies

[a] In general, the schedule and duration of administration in the toxicology study should mimic the clinical trial
[b] Should determine the dose severely toxic to 10% of the animals (STD_{10})
[c] Should determine toxicity of one-tenth the rodent STD_{10} on a mg/m2 basis
[d] One study should include histopathology
[e] Only for phase I testing in normal volunteers or patients believed to be disease-free

calculated from studies using the proposed clinical route, schedule, and duration.

The dose escalation scheme for phase I clinical studies often follows the standard or modified Fibonacci procedure [10]. Examples of other common and acceptable approaches include modified continual reassessment methods [13, 39] and pharmacokinetically guided dose escalation strategies [8]. These alternatives often necessitate a more extensive preclinical evaluation. For example, pharmacokinetic guidance of dose escalation is most effectively applied when: (a) linear pharmacokinetics are observed at drug concentrations spanning the pharmacological and toxicological effects, (b) the area under the drug concentration versus time curve (AUC) at the mouse STD_{10} can be defined, (c) protein binding in mouse and human plasma has been quantified, and (d) it is known whether metabolites contribute to the toxic effects [7, 8, 27, 40]. Although preclinical studies are used to determine the starting dose for phase I clinical trials, the highest doses for oncology drugs are rarely restricted by the doses used in preclinical toxicology studies as long as the toxicities of the new anticancer drug can be readily monitored, are reversible, and sufficiently precede lethality in animals. Instead, the maximum dose is restricted by the toxicity observed in the clinical trial, judged most often using NCI/DCTDC Common Toxicity Criteria [38].

Considerations for specific cytotoxic therapies

Combinations of cytotoxic agents

The evaluation of cytotoxic agent combinations has traditionally been conducted in the clinical setting using an empirical approach. This has generally been successful, but may not be optimal. Preclinical studies provide an opportunity to explore a variety of doses, dose ratios, and schedules to optimize benefit and minimize toxicity. Nonetheless, unless there is reason to believe that synergistic interactions occur that would substantially increase the toxicity of the combination, preclinical testing is not considered essential provided that each agent has been fully evaluated in humans. When synergistic effects may be anticipated such as when one agent interferes with the metabolism or elimination of the other agent or both cytotoxic agents target the same metabolic pathway or cellular function, preclinical testing of the combination is desirable.

Photosensitizers

One class of cancer chemotherapeutic drugs is therapeutically inactive until irradiated with light. These

photosensitizers or phototherapy agents usually form radicals after absorbing light energy that are ultimately responsible for tumor destruction. In photosensitizer therapy, tumor tissues are typically irradiated with laser light. When there is a choice, longer wavelengths of the irradiating light are preferred because they cause less direct tissue damage and because they penetrate more deeply into tumor tissue than shorter wavelengths.

Selective damage to tumor tissue is obtained by directing the activating light to the tumor. In addition, most phototherapy compounds concentrate in tumor tissues more than in surrounding normal tissue when given systemically. This increased concentration of photosensitizer combined with localized irradiation can kill tumor cells with great selectivity. Nevertheless, when these compounds are given systemically they commonly distribute in appreciable concentrations in all tissues and this provides the potential for toxicity. When these drugs accumulate in the eye or skin, patients may suffer irreversible retinal damage or severe phototoxicity similar to sunburn when exposed to ambient light [12]. Thus, it is important to know the plasma elimination half-life (and, if possible, tissue elimination half-lives) in preclinical studies so that the length of time a patient should protect themselves from light can be estimated.

Standard toxicity studies with multiple dose levels should be conducted in subdued illumination to clearly define the systemic toxicities of the photosensitizer. Subdued lighting allows systemic toxicities to be more clearly distinguished from phototoxicities. In addition to these standard toxicity studies, it is beneficial to assess phototoxicity before phase I clinical investigation begins because these drugs can cause prolonged photosensitivity. Acceptable models for these photosensitivity tests are either hairless or appropriately shaved species. The photosensitivity assessment should include toxicity testing as a function of both light dose (total energy) and drug dose and should ideally determine the duration of sensitivity in relation to plasma levels of the photosensitizer. Since a primary concern for the patient is the toxicity related to sunlight exposure, the light source for these tests should have a spectral distribution that approximates sunlight. Frequently, doses that are well below the no observable adverse effect limit (NOAEL) when the animal is housed in subdued light are lethal when the animal is briefly irradiated. Even though the photodynamic effect is expected to affect only tissues that are exposed to the light source, there is concern that photodegradation products could cause distant toxicities. Therefore, these phototoxicity tests usually include standard assessments of clinical signs, clinical pathology, gross pathology, histopathology of major organs, and the reversibility of toxicities. Clinical photodynamic therapy does not routinely involve repeated doses, and thus preclinical studies using daily irradiation during repeat dose testing may not be relevant to clinical safety concerns.

Without light these photosensitizers may not cause genotoxicity in standard tests, but subsequent irradiation may cause considerable damage to the DNA of cells exposed to the compound. Thus, genotoxicity tests are best done with and without light. The assessment of clastogenicity and mutagenicity should be done with increasing compound concentrations at a high light dose, and with increasing light dose (total energy) using broad-spectrum light at high compound concentration. The highest doses of drug of each series of tests should be consistent with international standards [19, 22].

In many cases an effective dose of drugs in this class is nontoxic in subdued light and the starting dose can be chosen based on efficacy studies rather than toxicity studies. This pertains only if the projected efficacious starting dose is lower than the safe dose estimated from the toxicity studies.

Specialized drug delivery

Administration of anticancer drugs as depots, attached to carriers, or in specialized encapsulated forms has the potential for significantly improving efficacy. Advantages of specialized drug delivery may include: (a) specific targeting of the drug to the tumor, (b) minimization of toxic side effects, (c) prolongation of therapeutic drug concentrations, (d) improved delivery of hydrophilic drugs to tumor cytoplasm, and (e) practical administration of very lipophilic drugs. Examples of delivery systems include copolymer implants, human albumin microspheres, monoclonal antibody–drug conjugates, and liposomal encapsulation. Development of anticancer drugs administered via carriers or in depots may necessitate additional preclinical evaluation beyond that of conventional cytotoxic drugs.

For antibody–drug conjugates, the two main safety concerns are the potential for toxicity from abrupt release of the drug and the potential for the antibody–drug conjugate to cause unexpected, specific toxicity in normal human tissues. Studies of the stability of the conjugate in human plasma as a function of the proposed release mechanism (e.g. pH if hydrolytic, glutathione concentration if reductive) help determine the necessity of conducting additional toxicology studies [21]. When additional studies are indicated, using the form of the drug released from the conjugate (i.e. including linker groups) may identify clinically important toxicities. Testing the reactivity of the conjugate with a complete panel of human tissues from at least three different sources is suggested [21]. When the target antigen is not expressed in the tissues of the standard preclinical animal models, a tolerance study in Pongidae apes at a dose that is at least double the planned human starting dose should also be considered. Both the reactivity screen and the tolerance study may reveal sites of potential tissue-specific toxicity, while the standard toxicology studies may define nonspecific toxicities. Specificity studies of binding or cytotoxicity in cell lines with and without an expressed target antigen also help to assess whether there is a significant differential between the toxicity to a targeted and nontargeted tissue. If feasible,

pharmacokinetic studies that distinguish between conjugate, free antibody, and free drug are also highly desirable for interpreting toxicology findings and supporting interspecies comparisons. Selection of a starting dose for clinical study should consider not only the results of the toxicity studies with the conjugate, but also the stability of the conjugate and the potential toxicity of released drug.

With liposomal drugs, standard preclinical toxicology studies of the delivery system, free drug, and the final formulation are important for evaluating a drug product's potential for toxicity. Liposomal formulations usually dramatically prolong systemic exposure. Thus, when repeated doses are to be used clinically, it is especially important to study a similar schedule preclinically because of the potential for drug accumulation. When the delivery system is designed to affect drug absorption, distribution, biotransformation, excretion or target organ accumulation, small changes in the design of the delivery system may have substantial effects on overall toxicity. Conducting the toxicity studies with the final formulation can avoid concerns about such effects. Comparative pharmacokinetic studies of the final formulation versus free drug can be very helpful in suggesting schedules and interpreting changes in the spectrum and severity of toxicities. Occasionally, studies of the empty liposomes plus free drug in combination may also be useful for understanding alterations in efficacy seen with the liposomal preparation. For example, blank liposomes may alter the pharmacokinetics of the free drug in a fashion sufficient for therapeutic gain [33].

Preclinical development of depot formulations generally follows that of liposomal formulations. Additionally, a study of the toxicity of the depot in the tissue or compartment intended to be used clinically should be conducted which includes a histopathologic examination of the adjacent tissues. Initial clinical doses similar to the total dose of the drug previously investigated in humans may be used in the absence of significant changes in toxicity profile for the depot formulation.

Alternative therapies

"Alternative" therapies include both single agents and multicomponent entities derived from plants or animals. Herbal products and tissue or fluid extracts from animal sources intended for the treatment or prevention of cancer or precancerous conditions belong in this category. The identity of the active ingredient of these entities is frequently uncertain. Consistency in taxonomic identification, collection, storage, and processing may pose additional difficulties. A useful initial step is to prepare a batch of the drug product large enough to be sufficient for both initial preclinical and clinical studies. The usual battery of toxicology studies for anticancer agents should be conducted unless there is adequate human safety experience. Since it is difficult to correlate specific drug product components with pharmacologic action, attempts should be made early in the development scheme to control the manufacturing processes to produce consistent batches for subsequent preclinical and clinical study. Further efforts should be made in the later stages of development to identify biologic assays which can be used to assure activity and as release specifications for the marketed product.

Herbal products represent a specialized subset of alternative therapies, as there is often significant human experience with their use. If there is a documented history of use of the herbals or if these preparations are freely marketed in the United States, then no preclinical pharmacology or toxicology is required for initial trials using the marketed product. Submission of data on the traditional use, preparation of the product, and safety profile of any known components of the herbal preparation for the IND is encouraged. When a product different from the marketed version is intended for the clinical trial, information on the preparation of the product to be tested is important in determining whether toxicology studies are necessary. If a herbal product is prepared in a manner different from the marketed product (e.g. alcoholic extraction instead of an aqueous preparation such as tea) or administered by an alternative route, then the standard toxicology studies for an investigational anticancer drug may be necessary. As the development of the herbal therapeutic agent continues in expanded trials, animal data including the histopathology, serum chemistry, hematology, reproductive, and genetic effects of the compound should be obtained either through literature data on the individual components of the herbal product or through toxicologic testing.

Considerations for chronic therapies

Chemopreventives

The preclinical development of chemopreventives has been previously described and should proceed similarly to most nononcology drugs [32]. The key considerations are summarized in Table 2.

Adjuvant therapy

The preclinical studies expected for drugs developed for adjuvant therapy depend on the prior human experience with the drug, the anticipated risks and benefits for the intended patients, and the expected mechanism of action. Few drugs are initially tested in humans in the adjuvant setting. Substantial clinical experience with these drugs is thus usual by the time they are considered for therapy in patients who have had their primary tumor removed or controlled. Nonetheless, further preclinical testing may be needed, depending on whether there are changes in the pattern of clinical use.

Additional preclinical studies that focus on long-term toxicity should be conducted for agents with which there is limited long-term clinical experience and intended for chronic treatment of patients in whom the risk of recurrence of cancer is relatively low. Cytotoxic drugs normally do not need additional long-term studies to support adjuvant use because the clinical experience with these drugs is usually extensive, they are usually administered using intermittent cycles rather than daily dosing, and the risks to patients are already well understood. When conducted, long-term studies should use the intended adjuvant route and schedule for at least as long as the intended clinical treatment duration, up to a maximal duration of 6 months in rodents and 12 months in nonrodents (usually dogs). A complete battery of genetic toxicity tests should be conducted prior to trials in patients believed to be free of disease. Carcinogenicity studies are usually expected prior to application for market approval.

Hormonal drugs

The mechanism of action of hormonal drugs differs significantly from that of other antineoplastic agents. These drugs are usually not directly cytotoxic, but may act as antiestrogens, progestins, antiprogestins, androgens, antiandrogens, aromatase inhibitors, or gonadotropin releasing hormone agonists. As with cytotoxic therapies, the preclinical toxicity assessment of hormonal drugs should use a similar route, schedule, duration of treatment, and formulation of drug substance as that proposed in clinical therapy. Standard 28-day toxicology studies with daily drug administration usually support small, phase I and phase II clinical trials with advanced-stage cancer patients. As clinical studies with longer durations of treatment are planned in patients likely to have an extended survival, additional preclinical testing usually follows the standard practice that the duration of the toxicology study be at least as long as the clinical trial. Because hormonal agents are generally used over an extended period, the complete toxicology assessment may need to focus on long-term effects on organ systems. Maximal duration of treatment in animals is usually limited to 6 months in rodents or 12 months in nonrodents. Although these agents are customarily developed for sex-specific indications, preclinical testing of both sexes allows identification of toxicities unrelated to the primary hormonal action of the drug that may be obscured in animals of the same sex as the intended treatment population. In addition, sex-based differences in the nonreproductive organ toxicities, sensitivity, or metabolism of a given drug may not be correlated across species [6]. Testing of hormonal agents in both sexes is thus more likely to provide the full spectrum of potential toxicities associated with a drug's use.

It is expected that the standard battery of genotoxicity tests assessing mutagenicity and clastogenicity will be conducted prior to phase I testing in patients believed to be disease-free [19, 22]. Carcinogenicity studies are expected if the hormonal drug is intended for use in patients believed to be disease-free or as adjuvant therapy. Studies should be conducted to evaluate reproductive performance and fertility in rats (ICH stage A–B, segment I), and teratogenicity in rats and rabbits (ICH stage C–D, segment II). Depending on the patient population and the duration of hormonal therapy, ICH stage C–F (segment III) studies may be needed. Many estrogen agonists or antagonists are structurally or pharmacodynamically related to diethylstilbestrol (DES), which is known to cause reproductive tract malignancy and abnormalities in humans exposed in utero [25]. Testing the potential of compounds related to DES to cause reproductive tract changes in neonates and pubescent animals is therefore considered important [5, 31, 44, 45]. Such studies typically focus on reproductive tract development following 3–5 days of dosing in rodent neonates in order to observe such pathologies as vaginal adenosis [44, 45].

Immunomodulators

Therapeutic agents that modulate the body's immune response to cancerous cells usually do so at concentrations significantly lower than those that cause severe toxicities in animals of the type seen with standard cytotoxic agents. Some biological responses to immunomodulators are species specific and may be related to toxicity to the immune system. Non-species-specific toxicities, however, do occur that are not directly related to modulation of the immune system. Thus, a standard safety evaluation conducted to identify these non-species-specific toxicities is important. As for hormonal agents, standard 28-day toxicology studies with daily drug administration are adequate to support initiation of phase I and phase II clinical trials that enroll advanced-stage cancer patients. In addition to the toxicology studies, knowledge of the mechanism of action also contributes to the evaluation of the safety of immunomodulators and selection of a starting dose. Studies that combine a measurement of the appropriate immunological response in addition to toxicity assessments are particularly useful because these agents, unlike most other drugs used to treat cancer, have sometimes exhibited bell-shaped dose response curves for desired activities. It is therefore especially important to use a starting dose that does not exceed the beneficial therapeutic range. The low doses often administered early in the phase I study sometimes give plasma concentrations of immunomodulators that preclude conventional pharmacokinetic study. In lieu of pharmacokinetic data, it may be useful to provide animal data on possible surrogate endpoints of activity that can be used in the clinic to demonstrate that active concentrations have been reached. Surrogate markers of activity that have been assessed include induction of interferon,TNF-α, neopterin, or β–2-microglobulin [41, 49].

Considerations for modulating therapies

Multidrug resistance-reversing agents

Prior to therapy, during therapy, or at the time of relapse, many tumors develop resistance to a variety of structurally unrelated anticancer drugs. This phenomenon is termed multidrug resistance (MDR). Mechanisms of MDR include, but are not limited to, altered expression of P-glycoprotein (P-gp), MDR-associated proteins (e.g. MRP and LRP), topoisomerases, and glutathione-*S*-transferases. Currently most MDR-reversing agents under development target the P-gp-dependent mechanism. P-gp is encoded by the *mdr*1 gene that is often amplified or overexpressed in MDR-manifesting tumors [26]. By functioning as an efflux pump, P-gp causes decreased drug accumulation and reduced cytotoxicity of anticancer drugs in tumor cells. P-gp is also expressed in many normal tissues (e.g. in the gastrointestinal tract, brain, kidney, and liver) [9]. One role of P-gp expression is presumably to carry out the efflux of toxic substances from these tissues. The inhibition of the efflux function of P-gp by a MDR-reversing agent increases intracellular concentrations of the cytotoxic drug in tumor tissue expressing P-gp. However, inhibition may also increase levels of cytotoxic drugs in normal P-gp-expressing tissues, potentially resulting in alterations of the severity and types of toxicities usually associated with the cytotoxic drug alone [1]. Furthermore, clinical and preclinical studies have shown that drugs interacting with P-gp can significantly alter the pharmacokinetics of cytotoxic drugs [2].

In view of the added risks associated with the combination of a MDR-reversing agent and a cytotoxic drug(s), the following preclinical studies are considered important for determining the safety of a proposed clinical trial. First, a standard profile of toxicology studies for the MDR-reversing agent alone should be conducted which take into consideration the likely duration of use in early clinical trials. Second, a study of the MDR-reversing agent combined with the cytotoxic drug in one species (usually a rodent) should be conducted to assess toxicity at both minimally and significantly toxic doses of the cytotoxic agent (Table 3). This information may also be derived from in vivo combination efficacy studies when an assessment of toxicity has been included. Based on experience to date, a combination study with one cytotoxic drug from a structurally related therapeutic class generally suffices for determining the safety of the modulator with all cytotoxic drugs in that class. Third, appropriate pharmacokinetic parameters should be derived since pharmacokinetic changes have often been shown to be important in interpreting the toxicity from such combinations.

There are several approaches for the selection of starting doses and escalation schemes for combinations of anticancer drugs and MDR-reversing agents. Some investigators have chosen to use a relatively high dose (or effective concentration) of MDR reverser and to escalate the anticancer drug. Others have started with a relatively high dose of the cytotoxic drug and escalated the MDR reverser. Neither of these approaches has been established as superior. Preclinical studies can guide either approach to dose selection by establishing a ratio of toxicity or potential toxicity of a given dose of the cytotoxic drug in the presence and absence of the MDR reverser. Acceptable endpoints for establishing this ratio might include direct measures of severe toxicity, such as marrow suppression or lethality, or measures of plasma concentrations. For example, when a therapeutic dose of a reversing agent increases the AUC of the cytotoxic drug fivefold in a preclinical model, a starting dose of the cytotoxic drug that is decreased by a factor of five from the accepted clinical dose of the cytotoxic drug alone would usually be appropriate. Further adjustments in the dose of the cytotoxic drug, either up or down, can then be derived from the initial clinical experience.

Radiation and chemotherapy sensitizers

Additional preclinical studies are usually important for the development of sensitizing agents for oncologic indications. In addition to the standard profile of toxicology studies in two species for the sensitizer alone, data on the ability of a sensitizing agent to enhance the toxicity of a cytotoxic or cytostatic therapy to non-neoplastic tissue is highly desirable. As for MDR-reversing agents, a study of the sensitizer combined with the cytotoxic therapy in one species (usually a rodent) that assesses toxicity at both minimally and significantly toxic doses of the cytotoxic agent or radiation therapy is considered important (Table 3). Although this is a straightforward toxicology study when the sensitizer is combined with a drug (e.g. L-buthionine-*S*,*R*-sulfoximine and alkylating agents), it is not so simple with radiosensitizers because radiation toxicity may only be apparent upon histopathologic examination and because the toxicity can be substantially delayed. One approach to address this issue with radiosensitizers is to conduct skin and leg contracture assays in mice [43] in lieu of comprehensive toxicology studies of the combination. The dosing scheme for these animals should be designed to support the planned clinical trial, but given the common clinical use of highly fractionated radiotherapy, this may not always be feasible.

How best to conduct the initial clinical trial is highly dependent on the combination modality, and advice on dose escalation and scheduling is product-specific. When the sensitizer is intended for combination with a therapy that has curative potential, the starting dose, frequency of dosing, and dose escalation plan for the new sensitizer needs to be carefully considered. Enhanced toxicities from the combination that significantly shorten or delay cycles of the standard therapy should be avoided so that efficacy of the standard therapy is maintained. One

accepted approach is to administer a full standard dose of radiation or anticancer agent, and a dose of the sensitizer projected to have some activity but that imparts little toxicity to the treatment regimen. Other approaches may also be acceptable provided that they are supported by a sound scientific rationale.

Chemoprotection

Chemoprotection is the use of drugs to mitigate the toxic effects of antineoplastic compounds. Marketed examples of this class include dexrazoxane, amifostine, mesna and leucovorin, which decrease the toxicities of doxorubicin (heart), cisplatin (kidney), ifosfamide (bladder) and methotrexate (high dose rescue), respectively. The toxicologic testing of the chemoprotective agent alone in one rodent and one nonrodent species should be based on the proposed use in the clinical trials. Usually these studies are done with a similar route, schedule and duration of administration as when combined with the antineoplastic agent. Reproductive toxicity testing for the protectant alone should be considered when the protectant is to be combined with a chemotherapeutic agent not known to be teratogenic. When the chemotherapeutic agent is known to be teratogenic, it may be useful to assess the ability of the protective agent to prevent this toxicity. The initial clinical dose for a chemoprotectant should ideally be chosen based on projected efficacy, but should not exceed the dose selected by standard toxicity criteria (i.e. one-tenth the rodent STD_{10} unless that dose is severely toxic to nonrodents).

While the primary issue is the toxicity of the chemoprotective agent alone, additional concerns include the possibility of protection of the tumor from the antineoplastic effects of chemotherapy and the possible augmentation of some of the toxic effects of the chemotherapeutic agent. For example, leucovorin, while able to mitigate the effects of an overdose of methotrexate, can also increase the toxicity of 5-fluorouracil [42]. Diethyldithiocarbamate, investigated to decrease the toxicity of cisplatin, actually increased the rate of tumor regrowth following the end of chemotherapy in a rat in vivo model when administered intraperitoneally [3]. In a clinical study, no change in response to 100 mg/m^2 cisplatin was noted, while the patient withdrawal due to toxicity was increased significantly in the diethyldithiocarbamate plus cisplatin arm [24]. In another clinical study, treatment with pyridoxine (vitamin B_6) to reduce the neurotoxicity of hexamethylmelamine and cisplatin was associated with a significant decrease in duration of response in ovarian cancer patients [48]. These clinical findings emphasize that the potential for tumor protective effects and changes in toxicity should be examined in preclinical studies of chemoprotective agents.

Toxicity data on the combination of the chemoprotectant and the antineoplastic agent can be derived from efficacy experiments, provided that histopathologic data are collected. Although in vitro data are useful, the influence on tumor protection by the chemoprotectant should be examined in vivo, where additional factors such as changes in metabolic profile of either drug may affect outcome. Comparisons of the duration of response (i.e. time to tumor regrowth) between an antineoplastic alone and the combination of antineoplastic and chemoprotectant are particularly important. This information on the interaction between the chemoprotective agent and the chemotherapeutic agent can be valuable for the design of pivotal or large scale clinical studies.

Considerations for changes in route or formulation

Changes in the route of administration or in the formulation of anticancer drugs are often pursued with a goal of improving drug utility. If a clinical trial is proposed by the oral route for a drug that has already been investigated by intravenous administration, then additional preclinical studies should address whether there is enhanced liver toxicity, direct gastrointestinal toxicity, or altered metabolism (due to microflora in the gastrointestinal tract, the intestinal wall, or a first-pass effect through the liver). An oral animal toxicity study with bioavailability data or an oral animal efficacy study with assessment of gastrointestinal and liver toxicity can address these concerns. The schedule of administration used in such a study should reflect the planned schedule of administration in the proposed phase I clinical study. A careful assessment of the forms of the drug present in blood should also be attempted, particularly if it is believed that metabolites contribute to the activity or toxicity. Pharmacokinetic information in humans for the i.v. formulation would also be useful for determining the starting dose of the oral formulation, but is not mandatory.

When i.v. administration is proposed for a drug with which there is oral clinical experience, the main concern is that the systemic exposure and resulting toxicity may be much greater by the i.v. route. Either an i.v. animal toxicity study (using the same schedule of administration as proposed for the initial phase I trial) or pharmacokinetic data with the oral formulation in humans that supports an acceptable exposure after the i.v. administration is important before beginning a trial with an i.v. formulation. Similarly, concerns about increased systemic exposure should be addressed when the formulation of an oral anticancer agent in clinical trials is changed. The studies needed to support use of the new formulation depend on the bioavailability of the original formulation in humans and on whether the potential exists to significantly increase bioavailability with the new formulation. For example, if the bioavailability in humans of the original formulation is near 100%, then there is little risk of increased toxicity with the new formulation and no new studies would be needed. On the other hand, if the bioavailability of the original formulation in humans is low, then a bioavailability

study comparing the new formulations in an appropriate species should be considered. An appropriate starting dose for the initial phase I trial with the new formulation can be projected from these data. In some circumstances, it may be sufficient to test a dose of a new formulation in humans without an animal study, so long as the dose is reduced to take into account potential changes in bioavailability.

Summary

Preclinical studies are an essential component of the drug development process. The preclinical development of new anticancer drugs is unique because of the life-threatening nature of the disease and because in most cases humans will be dosed to toxicity. In oncology, these studies are particularly useful in determining potentially safe and effective starting doses and schedules for a clinical trial. These studies also help to predict clinical toxicities and their reversibility, and provide a means for the determination of a dose-escalation scheme. The availability of adequate preclinical data can minimize the number of patients treated with ineffective doses or therapies in phase I trials and allow rapid determination of phase II doses. Preclinical studies are most useful when conducted using the same schedule, duration, formulation, and route of administration as that proposed in the clinical trial.

Basic research continues to provide information about new cellular mechanisms central to malignancy and often leads to drugs that attempt to exploit those mechanisms. The optimal development of a new class of drugs may differ from successful approaches used in the development of older well-established classes. New biological endpoints and new methods in toxicology may also be discovered and cannot be anticipated. The recommendations in this report have thus attempted to avoid being so restrictive and specific as to impede the development of innovative therapeutics for clinical use. Instead, the concerns that should be addressed have been emphasized.

It is assumed that most of the studies conducted to assess the toxicity profile of a drug follow GLP [16, 17]. Before a phase I clinical trial is initiated in patients with advanced cancers, two preclinical toxicity studies are usually conducted. One is a study in a rodent species that can identify doses that result in life-threatening and non-life-threatening toxicities. The other is a study to confirm that doses are identified that are not lethal and do not cause serious or irreversible toxicity in a nonrodent species. These studies, to the extent feasible, should be based on a rational schedule for efficacy and mimic the schedule and duration proposed in the phase I clinical trial.

Although not required, pharmacodynamic and pharmacokinetic studies can provide substantial additional support for the safety profile (starting dose, escalation, and drug combinations) and optimal potential use of the drug (tumor type, schedule, and route). This information is especially important in the development of noncytotoxic drugs (e.g. MDR reversers and immunomodulators) where the objective of the phase I or II clinical study may not be to reach MTD.

Depending on the type of antineoplastic agent under study, different approaches for estimating starting doses are appropriate. The phase I starting dose for cytotoxic agents in humans is generally one-tenth of the rodent STD_{10} on a milligrams per meter squared basis so long as this starting dose does not cause serious irreversible toxicities in nonrodents. If this dose causes irreversible toxicities in nonrodents, then the starting dose should be no more than one-sixth of the highest dose that does not produce lethality or serious irreversible toxicity in the nonrodent species. For noncytotoxic agents, starting dose selection should take into account the drug's pharmacodynamically active doses, provided that they do not cause substantial toxicity. Regardless of the method used to select the starting dose, the planned dose escalation scheme should be designed based on the slopes of the dose response curves for toxicodynamics and pharmacodynamics, the types of toxicities observed, and the pharmacokinetics of the drug.

In the later stages of anticancer drug development, when information is available on toxicity to humans, the need for additional toxicology studies should be evaluated. For most cytotoxic drugs, toxicity studies of limited duration suffice. With drugs intended for chemoprevention, adjuvant therapy, long-term hormone therapy, or long-term immunomodulator therapy, animal toxicity studies up to a maximum of 6 months in rodents and 12 months in a nonrodent species may be important for assessing safety and for supporting marketing approval. In addition, reproductive toxicity and carcinogenicity studies should be conducted when appropriate (e.g. for chemoprevention indications). Depending upon the nature of toxicity profiles in animal species and humans, special studies addressing potential organ system toxicities may also be useful.

The CDER Division of Oncology Drug Products of the FDA welcomes discussion of specific anticancer drugs at the early stages of development to facilitate rapid and efficient drug development. Prior to filing an IND, sponsors may have discussions with appropriate FDA staff and request pre-IND evaluations of their study plan. This may help sponsors to avoid spending time and resources on unnecessary studies, and may help to expedite initiation of clinical studies of promising new drugs.

Acknowledgements We thank Drs. Young Soo Choi, Misoon Chun, Tao Du, Lawrence F. Sancilio, and Virgil Whitehurst of the Division of Pulmonary Drug Products, FDA; Drs. Robert J. DeLap, Robert Justice, and the Medical Officers of the Division of Oncology Drug Products, FDA; and Dr. Robert J. Temple of the Office of Drug Evaluation I, FDA for their helpful comments during the preparation of this report.

References

1. Barbui T, Rambaldi A, Parenzan L, Zucchelli M, Perico N, Remuzzi G (1992) Neurological symptoms and coma associated with doxorubicin administration during chronic cyclosporin therapy. Lancet 339: 1421
2. Bartlett NL, Lum BL, Fisher GA, Brophy NA, Ehsan MN, Halsey J, Sikic BI (1994) Phase I trial of doxorubicin with cyclosporine as a modulator of multidrug resistance. J Clin Oncol 12: 835
3. Borch RF, Katz JC, Lieder PH, Pleasants ME (1980) Effect of diethyldithiocarbamate rescue on tumor response to cisplatinum in a rat model. Proc Natl Acad Sci USA 77: 5441
4. Branda RF (1981) Transport of 5-methyltetrahydrofolic acid in erythrocytes from various mammalian species. J Nutr 111: 618
5. Branham WS, Zehr DR, Chen JJ, Sheehan DM (1988) Alterations in developing rat uterine cell populations after neonatal exposure to estrogens and antiestrogens. Teratology 38: 271
6. Choudary J, Contrera JF, DeFelice A, DeGeorge JJ, Farrelly JG, Fitzgerald G, Goheer MA, Jacobs A, Jordan A, Meyers L, Osterberg R, Resnick C, Sun CJ, Temple RJ (1996) Response to Monro and Mehta proposal for use of single-dose toxicology studies to support single-dose studies of new drugs in humans. Clin Pharmacol Ther 59: 265
7. Collins JM, Zaharko DS, Dedrick RL, Chabner BA (1986) Potential roles for preclinical pharmacology in phase I clinical trials. Cancer Treat Rep 70: 73
8. Collins JM, Grieshaber CK, Chabner BA (1990) Pharmacologically guided phase I clinical trials based upon preclinical drug development. J Natl Cancer Inst 82: 1321
9. Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR (1990) Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. J Histochem Cytochem 38: 1277
10. DeVita VT Jr (1993) Principles of chemotherapy. In: DeVita VT Jr, Hellman S, Rosenberg SA (eds) Cancer: principles and practice. Lippincott, Philadelphia, p 276
11. Doroshow JH, Locker GY, Myers CE (1979) Experimental animal models of adriamycin cardiotoxicity. Cancer Treat Rep 63: 855
12. Dougherty TJ (1993) Photodynamic therapy. Photochem Photobiol 58: 895
13. Faries D (1994) Practical modifications of the continual reassessment method for phase I cancer clinical trials. J Biopharm Stat 4: 147
14. Food and Drug Administration (1994) International Conference on Harmonization. Guideline for detection of toxicity to reproduction for medicinal products. Fed Register 59: 48746
15. Food and Drug Administration (1995) International Conference on Harmonization. Toxicokinetics: the assessment of systemic exposure in toxicity studies. Fed Register 60: 11264
16. Food and Drug Administration (1995) Content and format of investigational new drug applications (INDs) for phase 1 studies of drugs, including well-characterized, therapeutic, biotechnology-derived products. Center for Drug Evaluation and Research, Rockville, Md.
17. Food and Drug Administration (1996) Good laboratory practice for nonclinical laboratory studies. 21 Code Federal Regulations 58: 265
18. Food and Drug Administration (1996) International Conference on Harmonization. Final guideline on the need for long-term rodent carcinogenicity studies of pharmaceuticals. Fed Register 61: 8154
19. Food and Drug Administration (1996) International Conference on Harmonization. Guidance on specific aspects of regulatory genotoxicity tests for pharmaceuticals. Fed Register 61: 18198
20. Food and Drug Administration (1996) International Conference on Harmonization. Draft guideline on testing for carcinogenicity of pharmaceuticals. Fed Register 61: 43297
21. Food and Drug Administration (1997) Points to consider in the manufacture and testing of monoclonal antibody products for human use. Center for Biologics Evaluation and Research
22. Food and Drug Administration (1997) International Conference on Harmonization. Genotoxicity: a standard battery for genotoxicity testing of pharmaceuticals. Fed Register 62: 16025
23. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE (1966) Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50: 219
24. Gandara DR, Nahhas WA, Adelson MD, Lichtman SM, Podczaski ES, Yanovich S, Homesley HD, Braly P, Ritch PS, Weisberg SR, Williams L, Diasio RB, Perez EA, Karp D, Reich SD, McCarroll K, Hoff JV (1995) Randomized placebo-controlled multicenter evaluation of diethyldithiocarbamate for chemoprotection against cisplatin-induced toxicities. J Clin Oncol 13: 490
25. Giusti RM, Iwamoto K, Hatch EE (1995) Diethylstilbestrol revisited: a review of the long-term health effects. Ann Intern Med 122: 778
26. Gottesman MM, Pastan I (1993) Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 62: 385
27. Graham MA, Workman P (1992) The impact of pharmacokinetically guided dose escalation strategies in phase I clinical trials: critical evaluation and recommendations for future studies. Ann Oncol 3: 339
28. Gralla EJ, Fleischman RW, Luthra YK, Stadnicki SW (1979) The dosing schedule dependent toxicities of adriamycin in beagle dogs and rhesus monkeys. Toxicology 13: 263
29. Grieshaber CK, Marsoni S (1986) Relation of preclinical toxicology to findings in early clinical trials. Cancer Treat Rep 70: 65
30. Hard GC, Iatropoulos MJ, Jordan K, Radi L, Kaltenberg OP, Imondi AR, Williams GM (1993) Major difference in the hepatocarcinogenicity and DNA adduct forming ability between toremifene and tamoxifen in female Crl:CD(BR) rats. Cancer Res 53: 4534
31. Iguchi T, Hirokawa M, Takasugi N (1986) Occurrence of genital tract abnormalities and bladder hernia in female mice exposed neonatally to tamoxifen. Toxicology 42: 1
32. Kelloff GJ, Johnson JR, Crowell JA, Boone CW, DeGeorge JJ, Steele VE, Mehta MU, Temeck JW, Schmidt WJ, Burke G, Greenwald P, Temple RJ (1995) Approaches to the development and marketing approval of drugs that prevent cancer. Cancer Epidemiol Biomarkers Prev 4: 1
33. Kim S, Kim DJ, Howell SB (1987) Modulation of the peritoneal clearance of liposomal cytosine arabinoside by blank liposomes. Cancer Chemother Pharmacol 19: 307
34. Lelieveld P, Vijgh WJF van der, Velzen D van (1987) Preclinical toxicology of platinum analogues in dogs. Eur J Cancer Clin Oncol 23: 1147
35. Lowe MC, Davis RD (1984) The current toxicology protocol of the National Cancer Institute. In: Helmann K, Carter SK (eds) Fundamentals of cancer chemotherapy. McGraw-Hill, New York, p 228
36. Mettler FP, Young DM, Ward JM (1977) Adriamycin-induced cardiotoxicity (cardiomyopathy and congestive heart failure) in rats. Cancer Res 37: 2705
37. Montandon F, Williams GM (1994) Comparison of DNA reactivity of the polyphenylethylene hormonal agents diethylstilbestrol, tamoxifen and toremifene in rat and hamster liver. Arch Toxicol 68: 272
38. National Cancer Institute (1993) Investigator's handbook, Bethesda, Md.
39. O'Quigley J, Pepe M, Fisher L (1990) Continual reassessment method: a practical design for phase I clinical trials in cancer. Biometrics 46: 33
40. Peck CC, Barr WH, Benet LZ, Collins J, Desjardins RE, Furst DE, Harter JG, Levy G, Ludden T, Rodman JH, Sanathanan L, Schentag JJ, Shah VP, Sheiner LB, Skelly JP, Stanski DR, Temple RJ, Viswanathan CT, Weissinger J, Yacobi A (1992) Opportunities for integration of pharmacokinetics, pharmacodynamics, and toxicokinetics in rational drug development. Clin Pharmacol Ther 51: 465
41. Philip PA, Rea D, Thavasu P, Carmichael J, Stuart NSA, Rockett H, Talbot DC, Ganesan T, Pettit GR, Balkwill F,

Harris AL (1993) Phase I study of bryostatin 1: assessment of interleukin 6 and tumor necrosis factor alpha induction in vivo. J Natl Cancer Inst 85: 1812
42. Poon MA, O'Connell MJ, Wieand HS, Krook JE, Gerstner JB, Tschetter LK, Levitt R, Kardinal CG, Maillard JA (1991) Biochemical modulation of fluorouracil with leucovorin: confirmatory evidence of improved therapeutic efficacy in advanced colorectal cancer. J Clin Oncol 9: 1967
43. Stone HB (1984) Leg contracture in mice: an assay of normal tissue response. Int J Radiat Oncol Biol Phys 10: 1053
44. Taguchi O (1987) Reproductive tract lesions in male mice treated neonatally with tamoxifen. Biol Reprod 37: 113
45. Taguchi O, Nishizuka Y (1985) Reproductive tract abnormalities in female mice treated neonatally with tamoxifen. Am J Obstet Gynecol 151: 675

Note added in proof The draft ICH S4 document "Duration of chronic toxicity testing in animals (rodent and nonrodent toxicity testing)" is under consideration by the EU, Japan, and US. If implemented in its current form, the maximum duration of toxicity testing for nonrodents would change from 12 months to 9 months for most drugs undergoing international development.

46. Taudy M, Syka J, Popelar J, Ulehlova L (1992) Carboplatin and cisplatin ototoxicity in guinea pigs. Audiology 31: 293
47. US Interagency Staff Group on Carcinogens (1986) Chemical carcinogens: a review of the science and its associated principles. Environ Health Perspect 67: 201
48. Wiernik PH, Yeap B, Vogl SE, Kaplan BH, Comis RL, Falkson G, Davis TE, Fazzini E, Cheuvart B, Horton J (1992) Hexamethylmelamine and low or moderate dose cisplatin with or without pyridoxine for treatment of advanced ovarian carcinoma: a study of the Eastern Cooperative Oncology Group. Cancer Invest 10: 1
49. Witt PL, Ritch PS, Reding D, McAuliffe TL, Westrick L, Grossberg SE, Borden EC (1993) Phase I trial of an oral immunomodulator and interferon inducer in cancer patients. Cancer Res 53: 5176

VOLUME 23 · NUMBER 24 · AUGUST 20 2005

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Phase I Trial of the Oral Antiangiogenesis Agent AG-013736 in Patients With Advanced Solid Tumors: Pharmacokinetic and Clinical Results

Hope S. Rugo, Roy S. Herbst, Glenn Liu, John W. Park, Merrill S. Kies, Heidi M. Steinfeldt, Yazdi K. Pithavala, Steven D. Reich, James L. Freddo, and George Wilding

From the University of California, San Francisco Comprehensive Cancer Center, San Francisco; Pfizer Global Research and Development, San Diego, CA; University of Wisconsin Comprehensive Cancer Center, Madison, WI; The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Submitted November 7, 2004; accepted March 24, 2005.

Research support provided by Pfizer Global Research and Development, San Diego, CA, and Groton, CT.

Presented at the American Association for Cancer Research-National Cancer Institute-European Organisation for Research and Treatment of Cancer International Conference on Molecular Targets and Cancer Therapeutics, 2003, Boston, MA; 6th International Symposium on Antiangiogenesis Agents, 2004, San Diego, CA; and American Society of Clinical Oncology Annual Meeting, 2004, New Orleans, LA.

Authors' disclosures of potential conflicts of interest are found at the end of this article.

Address reprint requests to George Wilding, MD, University of Wisconsin Comprehensive Cancer Center, K4/610 CSC, 600 Highland Ave, Madison, WI 53792; e-mail: gxw@medicine.wisc.edu.

0732-183X/05/2324-5474/$20.00

DOI: 10.1200/JCO.2005.04.192

ABSTRACT

Purpose
We studied the safety, clinical activity, and pharmacokinetics (PK) of AG-013736, an oral receptor tyrosine kinase inhibitor of vascular endothelial cell growth factor, platelet-derived growth factor, and c-Kit, in patients with advanced cancer.

Patients and Methods
Patients received fixed doses of AG-013736 orally in 28-day cycles. In the first cohort, patients initially received two single test doses of AG-013736 (10 and 30 mg); subsequent dosing was determined by individual PK parameters. Doses in subsequent cohorts were assigned by using a traditional dose-escalation/de-escalation rule based on observed toxicities in the current and previous cohorts. PK analysis included evaluation of the effect of food and antacid.

Results
Thirty-six patients received AG-013736 at doses ranging from 5 to 30 mg by mouth twice daily. The dose-limiting toxicities observed included hypertension, hemoptysis, and stomatitis and were seen primarily at the higher dose levels. The observed hypertension was manageable with medication. Stomatitis was generally tolerable and managed by dose reduction or drug holidays. AG-013736 was absorbed rapidly, with peak plasma concentrations observed within 2 to 6 hours after dosing. The maximum-tolerated dose and recommended phase II dose of AG-013736 is 5 mg, twice daily, administered in the fasted state. No significant drug interaction with antacid was seen. There were three confirmed partial responses and other evidence of clinical activity.

Conclusion
In this study, we have demonstrated clinical activity and safety of AG-013736 in patients with advanced solid tumors and identified the dose for phase II testing. The unique phase I study design allowed early identification of important absorption and metabolic issues critical to phase II testing of this agent.

J Clin Oncol 23:5474-5483. © 2005 by American Society of Clinical Oncology

Introduction

Angiogenesis is necessary for the progression from benign to malignant tumors, as well as growth and metastases of malignant cells.[1-7] Disruption of endothelial cell responses responsible for abnormal blood vessel formation may be used to stop tumor growth.[8-11]

A strong correlation has been observed between vascular endothelial cell growth factor (VEGF) expression and tumor microvessel density in multiple tumor types,[12-14] supporting the role of VEGF in facilitating tumor angiogenesis[15,16] through its receptors. Abrogation of signaling through both the VEGF receptor (VEGFR) and platelet-derived growth factor receptor-β (PDGFR-β) has been

demonstrated to inhibit angiogenesis and tumor growth in preclinical models, with early supportive clinical results.[17-20]

AG-013736 is a substituted indazole derivative that was discovered by using a structure-based drug design. Mechanistically, AG-013736 is a potent small molecule tyrosine kinase inhibitor of all known VEGFRs at subnanomolar concentrations and PDGFR-β and c-Kit in low nanomolar concentrations. In vitro, AG-013736 selectively blocks VEGF-stimulated receptor autophosphorylation leading to inhibition of endothelial cell proliferation and survival. In mice, AG-013736 inhibited tumor vascular angiogenesis and the growth of human colorectal and murine lung tumors. This antitumor effect was associated with a significant decrease in microvessel density and increased tumor necrosis. In a transgenic mouse model of spontaneous islet cell tumors, treatment with AG-013736 eliminated endothelial fenestrations and suppressed vascular sprouting within 24 hours. At 7 days, vascular density decreased more than 70%, and significant tumor shrinkage was seen at 21 days.[21]

Given strong preclinical evidence for antitumor/antimetastatic activity, a first-in-human phase I trial was conducted to test AG-013736 in patients with advanced solid malignancies. The primary objective was to determine dose-limiting toxicity (DLT) and the maximum-tolerated dose (MTD) of AG-013736. Secondary objectives were to (1) evaluate the pharmacokinetics (PK) of oral AG-013736, (2) conduct a pilot evaluation of the effect of food on AG-013736 PK, (3) conduct a pilot evaluation of the effect of an antacid on the PK of AG-013736, and (4) document preliminary evidence of antitumor activity. Dynamic contrast-enhanced (DCE) imaging was performed to evaluate the association between PK and vascular response; these data are reported in a separate publication.[22]

Patients and Methods

Patients were enrolled at the University of Wisconsin Comprehensive Cancer Center, University of Texas M.D. Anderson Cancer Center, and University of California, San Francisco Comprehensive Cancer Center between May 2002 and January 2004. All patients were $\geq$ 18 years of age with histologically confirmed advanced nonhematologic cancer refractory to standard therapy or for which no effective therapy was available. An Eastern Cooperative Group performance status of 0 to 2 and a life expectancy of $\geq$ 12 weeks were required. All patients had completed prior therapies with adequate time allowed for recovery from toxicity. Additional inclusion criteria included: adequate bone marrow, renal, and liver function (bilirubin $\leq$ 1.5 mg/dL, transaminases $\leq$ 2.5 $\times$ upper limit of normal or $\leq$ 5 $\times$ upper limit of normal with documented liver metastasis). No prior history of coronary artery disease, significant gastrointestinal abnormalities, or uncontrolled brain metastases was permitted. Patients with a positive stool guaiac test without evidence of active bleeding were included provided monitoring of the hemoglobin was performed during the trial. In addition, patients requiring the use of potent CYP3A4 inhibitors or CYP3A4/CYP1A2 inducers, chronic H_2 antagonists or proton-pump inhibitors, or therapeutic anticoagulant therapy were not eligible. By amendment, patients with proteinuria $\geq$ 500 mg/24 hours, squamous cell lung cancer, centrally located lung lesions (of any histology), and uncontrolled hypertension (systolic $>$ 140 mmHg, diastolic $>$ 90 mmHg) were excluded because of observed toxicities.

Drug Administration and Study Design

AG-013736 was supplied as 1- and 10-mg film-coated tablets. AG-013736 was administered either once daily (QD) or BID, with no scheduled breaks. Each cycle of therapy was defined as 28 days.

Calculation of Starting Dose and Continuous Drug Dosing

Initial dosing was chosen based on a modification of the recommendations of DeGeorge et al[23]: one sixth of the body-surface area–adjusted minimal effect dose (MED; 10 mg/kg/d) identified in a 28-day dog study of AG-013736. This resulted in a calculated safe human starting dose of 30 mg BID. Cohorts of 6 patients were planned at each dose level. To obtain an early evaluation of in vivo PK and safety, patients in cohort 1 were initially treated with a single test dose of 10 mg, followed by a single test dose of 30 mg at least 48 hours later. The two single doses were selected to not exceed the projected starting dose and to provide an early assessment of PK dose proportionality.

Each patient in the first cohort was then assigned a continuous dose based on their single-dose PK to obtain steady-state plasma exposures similar to that associated with the MED in dogs. The specific continuous dose was estimated as the product of observed apparent plasma clearance and target MED exposure in dogs. The six patients in cohort one received doses between 10 mg QD and 30 mg BID.

Based on the overall safety profile of the first cohort, the average dose evaluated in the first cohort (20 mg BID) was selected as the continuous dose for the second cohort. In subsequent cohorts, escalation of doses by 100% or 40% was planned. However, because of the significant toxicity seen in the first two cohorts, a dose de-escalation schema was used.

Dose Reductions and Modifications

Dose reductions or interruptions of AG-013736 treatment were added by amendment for hypertension, proteinuria, and hemoptysis. Dose modifications for other toxicities were decided between the investigator and sponsor. Intrapatient dose escalation was not allowed. Treatment was discontinued for disease progression, unacceptable toxicity, or withdrawal of consent. Patients were considered to have completed the study after receiving eight cycles of treatment; however, they could continue to receive AG-013736 on a separate follow-on protocol for as long as they derived benefit.

Definition of DLT/MTD

DLT was defined as any of the following during the first cycle when considered related to AG-013736 treatment: select grade 2 or higher gastrointestinal toxicity despite the use of medical intervention and/or prophylaxis, grade 3 anemia, grade 4 thrombocytopenia, or grade 3 nonhematologic toxicities except nausea, vomiting, diarrhea, constipation, pain, and hypertension controlled with medication. The MTD was defined as the dose at which no more than one of six patients in a single cohort experienced DLT in the first cycle.

PK

Plasma samples were collected over 12 hours after dosing in all patients for evaluation of multiple-dose PK on days 1 and 15 (cycle 1) and day 29 (cycle 2, day 1). Additionally, predose trough samples were collected on days 43 (cycle 2, day 15) and 57 (cycle 3, day 1). Whole blood (5 mL) was collected in K_3-EDTA vacutainer tubes at predose, 0.5, 1, 2, 4, 8, and 12 hours after dosing and centrifuged at 1,000 × *g* for 15 minutes, and plasma was frozen at −20°C. Analysis was conducted at Charles River Discovery and Development Services (Worcester, MA). Adequate precautions were taken to minimize degradation of AG-013736 in plasma/whole blood when exposed to visible light.

Assay for Measurement of AG-013736 in Human Plasma

Plasma concentrations of AG-013736 were measured by using a validated liquid chromatography-tandem mass spectrometric method (lower limit of quantitation, 0.1 ng/mL). Briefly, AG-013736 and the deuterated internal standard were extracted from plasma with an ethyl acetate/hexanes mixture. The extracts were evaporated to dryness, reconstituted in aqueous methanol mobile phase, and eluted from a reversed-phase column onto a triple-quadrupole mass spectrometer for detection.

Standard AG-013736 plasma PK parameters were estimated by using noncompartmental methods (using the WINNonlin Professional software; Scientific Consultant, Apex, NC), version 3.2. AG-013736 levels were also measured in the urine of patients.

Evaluation of Food Effect

Patients in cohorts two through four participated in a pilot food-effect study. On day 29, patients received their morning AG-013736 dose within 30 minutes of completion of a 1,000-calorie meal (50% caloric content from fat). On day 30, patients received their morning dose after an overnight fast of at least 6 hours. PK samples were collected for 12 hours on both days. Based on this evaluation, patients in the fifth and sixth cohorts were asked to fast for 2 hours before and after each AG-013736 dose.

Evaluation of Antacid Effect

Patients in cohort six participated in a pilot antacid-effect study to evaluate the effect of the proton-pump inhibitor rabeprazole (Aciphex) on AG-013736 PK. On days 30 to 34, patients received 20 mg of rabeprazole daily 3 hours before their morning AG-013736 dose. On day 34, PK samples were collected for 12 hours after the morning AG-013736 dose.

Pretreatment and Follow-Up Evaluations

Complete medical history, physical examination including vital signs, Eastern Cooperative Oncology Group performance status, full blood count, and serum chemistry and urinalysis were performed at baseline, weekly during the first cycle, and monthly thereafter. A 12-lead ECG and stool guaiac test were performed at baseline. A serum or urine pregnancy test was performed in women of childbearing potential. Toxicity was evaluated by clinical and laboratory examination and graded by using the National Cancer Institute Common Toxicity Criteria, version 2.0.[24] After hypertension was identified as a DLT, all patients were required to monitor and record their blood pressures at least once daily. Radiological or physical assessment of tumors was done within 28 days before the first dose and then repeated every two cycles (every 8 weeks). Patients were evaluated for response by using standard Response Evaluation Criteria in Solid Tumors (RECIST).[25]

Additional Assessments

Plasma samples were collected to evaluate exploratory biomarkers related to the VEGFR pathway. In an effort to evaluate a possible renal etiology for the hypertension seen in patients treated with AG-013736, renin, angiotensin II, and aldosterone levels were measured in the plasma of patients from cohorts five and six receiving 5 mg of AG-013736 BID in the fasted state. Blood samples were collected before dosing on day 1 and then 4 hours after the morning AG-013736 dose on days 1, 15, and 29 (cycle 2, day 1).

RESULTS

Patient Characteristics

A total of 36 patients with advanced solid malignancies were treated with AG-013736 in this phase I trial. The patients received oral doses ranging from 5 mg BID to 30 mg BID. The first four cohorts were dosed in the fed state, and the last two cohorts were dosed in the fasted state (defined as no food or drink other than water in the 2 hours before and after AG-013736 dosing; Table 1) after PK data demonstrated improved absorption in the fasted state (see PK). Selected patient characteristics are summarized in Table 2. Twenty-six patients (72%) had received prior chemotherapy, and 18 patients (50%) had prior radiotherapy.

PK

AG-013736 administered in the fed state was absorbed rapidly, with peak plasma concentrations occurring within 2 to 6 hours after dosing (Table 3, Fig 1). Plasma concentrations declined with a terminal plasma half-life between 2 and 5 hours. AG-013736 plasma PK reached steady state within 15 days, with no unexpected accumulation. Patients in cohorts two through four demonstrated generally linear PK, as evidenced by dose-proportional increases in maximum plasma concentration (C_{max}) and area under the curve (AUC; Fig 2).

At the phase II dose of 5 mg BID given in the fasted state, the between-patient coefficient of variation for $AUC_{0\text{-}24}$ on cycle 1, day 15 was 90% (Table 3); the corresponding coefficient of variation for C_{max} was 63%. A total

Table 1. Summary of Cohorts (N = 36)

Cohort	AG-013736 Dose	Fed/Fasted	No. of Patients
1	Various*	Fed	6
2	20 mg BID	Fed	4
3	5 mg BID	Fed	6
4	15 mg QD	Fed	6
5	5 mg BID	Fasted	8
6	2 mg BID (days 1-2) 5 mg BID (days 3+)	Fasted	6

Abbreviation: QD, once daily.
*The following doses were administered: 10 mg QD (one patient), 10 mg BID (two patients), 20 mg BID (two patients), and 30 mg BID (two patients).

Table 2. Characteristics of the 36 Enrolled Patients

Characteristic	Patients	
	No.	%
Age, years		
Median	57	
Range	41-76	
Sex		
Female	20	56
Male	16	44
ECOG performance status		
0	6	17
1	27	75
2	3	8
Race		
White	30	83
Asian	3	8
Hispanic	2	6
Black	1	3
Tumor type		
Breast	13	36
Renal cell	6	17
Thyroid	5	14
Mesothelioma	3	8
NSCLC	2	5
Colon	2	5
SCC skin	2	5
Other*	3	8

Abbreviations: ECOG, Eastern Cooperative Group; NSCLC, non–small-cell lung cancer; SCC, squamous cell carcinoma.
*Other tumor types included one patient each with prostate cancer, adenoid cystic carcinoma, and melanoma.

of 9 of 16 patients in cohorts two through four completed the pilot food-effect study. Both the rate and extent of absorption were higher in the fasted state, as evidenced by higher C_{max}, higher AUC_{0-24}, and a shorter T_{max} in the majority of patients. Peak concentrations occurred 1 to 2 hours after dosing in the fasted state, and there was a median 49% increase in plasma exposures compared with the fed state. Plasma half-life was not changed appreciably in fed and fasted states (Table 3, Fig 3). There was no difference in the effect of food across dose groups.

In the presence of the potent proton-pump inhibitor rabeprazole, the rate of absorption of AG-013736 was decreased (reduction in C_{max}), but the extent of absorption was unaffected (marginal to negligible changes in the AUC; Fig 4). Rabeprazole was administered in this pilot evaluation for 5 days to maximize its gastric pH-lowering effect. Because of the minimal change in plasma exposure in the presence of rabeprazole, the effect of antacids on AG-013736 absorption was not considered to be clinically significant.

Twelve-hour urinary collections obtained on days 1 and 29 (cycle 2, day 1) demonstrated that $< 1\%$ of the administered dose appeared as unchanged drug in the urine regardless of dose, which indicates that the majority of drug elimination was through systemic metabolism.

Soluble plasma proteins (VEGF, fibroblast growth factor, tumor necrosis factor-alpha, and matrix metalloproteinases 2 and 9) evaluated in this study as exploratory markers related to VEGFR signal transduction pathways did not show any variation with treatment.

DLT and MTD

The primary DLT was hypertension. All DLTs are summarized in Table 4. Based on the experience of the first two cohorts, a dose of 20 mg BID exceeded the MTD. DLTs also occurred in patients who were treated in the first two cohorts with 10 mg BID; consequently, this dose was considered to be above MTD and was never tested in a separate cohort.

The MTD and recommended phase II dose of AG-013736 is 5 mg BID in the fasted state. Of 14 patients treated in two cohorts with this dose, two DLTs (grade 2 stomatitis and grade 3 diarrhea) were observed (Table 4).

Safety

Toxicities reported in at least 10% of patients and all grade 3 and 4 toxicities are summarized in Table 5. The principal toxicities were hypertension, fatigue, diarrhea, stomatitis, nausea, and vomiting. Of these, hypertension was reported the most frequently, occurring in 22 patients (61%). The majority of cases (18 patients) were controlled easily with antihypertensive medications. Of the remaining four patients, two developed grade 3 or 4 hypertension and withdrew from the study without attempting medical management. The other two patients developed grade 1/2 hypertension not requiring medication and withdrew from the study early because of disease progression.

The incidence and severity of hypertension was dose dependent. In the first two cohorts in which doses ranged from 10 mg QD to 30 mg BID, hypertension was observed in all 10 patients and was grade 3 or 4 in severity in five patients. Seizures occurred in two patients in the first cohort (before hypertension was monitored and treatment guidelines were established): one patient at 20 mg BID and one at 10 mg BID. Neither of these patients had brain metastases or a prior history of seizures; both patients recovered without sequelae. In subsequent cohorts of patients receiving doses < 10 mg BID ($n = 26$), hypertension was a DLT in one patient receiving 15 mg QD. This patient continued on treatment at a lower dose with concurrent antihypertensive medication with good blood pressure control. Eleven other patients developed hypertension (including six of the 14 patients treated at the recommended phase II dose) that was not dose limiting and was managed by standard antihypertensive medications. No consistent shifts in the levels of renin, angiotensin II, and aldosterone were seen with drug treatment, suggesting that AG-013736–related hypertension is unlikely to be mediated through alteration of the renin-angiotensin-aldosterone pathway.

Table 3. Mean (percent coefficient of variation) AG-013736 Plasma Pharmacokinetic Parameters

	C_{max}, ng/mL	T_{max}, hours	AUC_{0-24}, ng · h/mL	$t_{1/2}$, hours	CL/F (L/h)	Vz/F (L)
2 mg BID, fasted						
Day 1 (n = 6)*	26 (70)	1.7 (72)	183 (80)	2.8 (72)	57 (139)	155 (83)
5 mg BID, fed						
Day 1 (n = 6)	22 (42)	3.2 (42)	188 (39)	2.3 (28)	56 (34)	188 (39)
Day 15 (n = 6)	27 (36)	2.4 (59)	258 (39)	2.3 (13)	45 (44)	145 (42)
Day 29 (n = 6)	31 (49)	4.2 (97)	380 (56)	2.8 (29)	36 (66)	148 (49)
5 mg BID, fasted						
Day 1 (n = 8)†	37 (93)	1.9 (70)	194 (72)	1.7 (34)	113 (106)	263 (108)
Day 15 (n = 12)‡	63 (66)	1.6 (54)	451 (94)	3.2 (101)	45 (92)	174 (137)
Day 29 (n = 12)‡	63 (57)	1.8 (68)	466 (85)	3.1 (26)	43 (113)	182 (134)
15 mg QD, fed						
Day 1 (n = 6)	108 (72)	2.7 (38)	668 (66)	3.4 (34)	35 (74)	180 (82)
Day 15 (n = 5)	82 (57)	6.0 (66)	717 (72)	4.6 (95)	51 (127)	215 (88)
Day 29 (n = 4)	106 (108)	3.0 (32)	712 (98)	2.7 (48)	56 (92)	174 (92)
20 mg BID, fed						
Day 1 (n = 6)	146 (63)	4.0 (55)	1,802 (65)	3.3 (39)	41 (134)	129 (60)
Day 15 (n = 4)	162 (39)	3.3 (46)	2,171 (30)	4.8 (58)	20 (30)	131 (50)

Abbreviations: BID, twice daily; QD, once daily; C_{max}, maximal plasma concentration during the dosing interval; T_{max}, time of maximal plasma concentration; AUC_{0-24}, area under the plasma concentration *v* time curve from 0 to 24 hours (estimated as twice the AUC_{0-12} for BID dosing); $t_{1/2}$, plasma elimination half-life; CL/F, oral plasma clearance; Vz/F, apparent volume of distribution of the drug during the elimination phase.

*Patients in cohort six, who received 2 mg BID during the first two days on the study, followed by 5 mg BID dosing for the remaining duration of the study.

†Patients in cohort five, who received 5 mg BID dosing.

‡Includes patients in cohorts five and six.

A variety of grade 1 to 2 mouth-pain events (eg, stomatitis, glossodynia) were reported as related to AG-013736 in 11 patients. Two patients experienced grade 3 stomatitis, accompanied by grade 3 throat pain in one patient. Dose reductions or drug holidays helped to manage this toxicity. Grade 1 hoarseness or voice changes were reported as a drug-related event in five patients.

Drug-related diarrhea was generally grade 1 to 2: one patient in cohort six experienced intermittent grade 3 diarrhea and required dose reduction. Loperamide was used successfully to manage diarrhea in most patients. Nausea and vomiting were grade 1 to 2 in severity and manageable with medications.

Five patients died during the study; three of the deaths were related to disease progression. Two patients with adenocarcinoma of the lung (non–small-cell lung cancer) in the second cohort (20 mg BID, reduced to 10 mg BID) developed fatal hemoptysis. One patient had a centrally located lung lesion and died acutely with grade 4 hemoptysis while on AG-013736. Treatment with high-dose AG-013736 likely led to the rapid breakdown of a vessel wall with tumor infiltration, followed by fatal hemoptysis. The second patient with a peripherally located lung lesion developed grade 1 hemoptysis while on AG-013736; consequently, treatment with the study drug was discontinued. Two weeks later, the patient died of lung complications including grade 4 hemoptysis, which was ultimately determined by the investigator to be related to disease and concurrent infection. Based on these episodes of serious hemoptysis as well as reported similar bleeding events seen with other antiangiogenic agents, the protocol was amended to exclude patients with centrally located lung lesions of any type as well as patients with squamous cell carcinoma of the lung. The only other bleeding event reported as related to AG-013736 was one case of grade 1 rectal bleeding.

Abnormalities in laboratory parameters were reported as adverse events in Table 3 if they resulted in dose reduction, treatment interruption, or clinical sequelae or were otherwise considered important by the investigator. Findings from review of the laboratory data are summarized below.

Asymptomatic proteinuria was observed in 7 of the 10 patients enrolled in the first two cohorts, including two patients with grade 3 proteinuria by dipstick. The protocol was subsequently amended to exclude patients with proteinuria $>$ 500 mg over 24 hours; dose adjustment or suspension of AG-013736 dosing was required for patients with proteinuria $\geq$ 1 g over 24 hours. In subsequent cohorts treated at lower doses of AG-013736, proteinuria was less frequent and less severe. Of these 26 patients, only six developed grade 1 or 2 proteinuria after starting treatment.

Three patients developed grade 3 transaminase elevations while receiving AG-013736. One patient with metastatic breast cancer developed pancreatitis and ischemic bowel associated with superior mesenteric vein thrombosis on15 mg QD. The cause of this constellation of events was not determined, although tumor infiltration was suspected. In one patient, the change in liver-function tests was reported

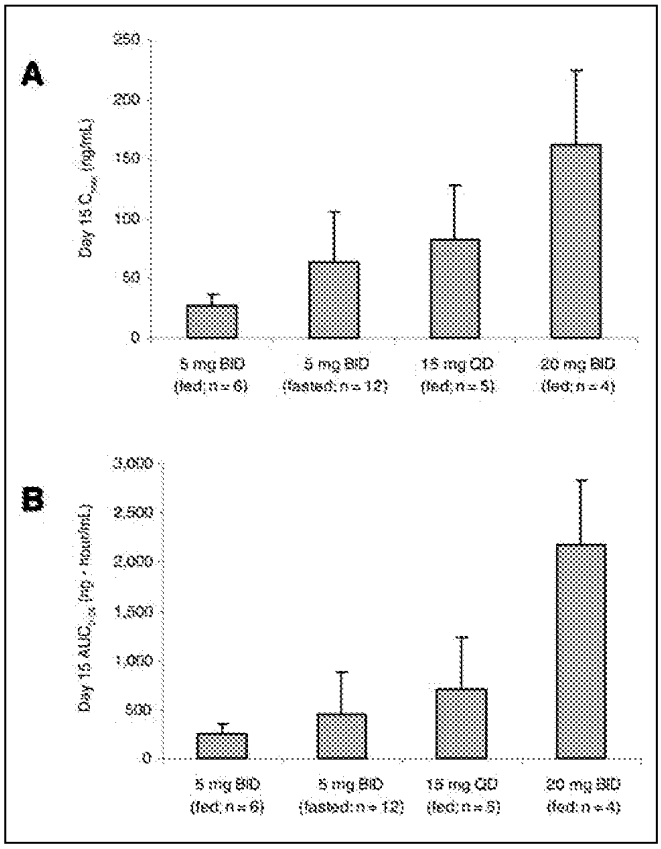

Fig 1. Mean steady-state AG-013736 plasma concentrations on day 15 of dosing. (A) C_{max}, maximum concentration; (B) AUC, area under the curve. QD, every day.

to be related to a concomitant medication (enalapril). The third case occurred in a patient receiving 20 mg BID.

There was only one notable hematologic toxicity at any dose level: grade 2 thrombocytopenia resulting in treatment discontinuation in a patient taking 20 mg BID.

Efficacy

The primary purpose of this study was to establish the safety and MTD of AG-013736. In addition, patients were

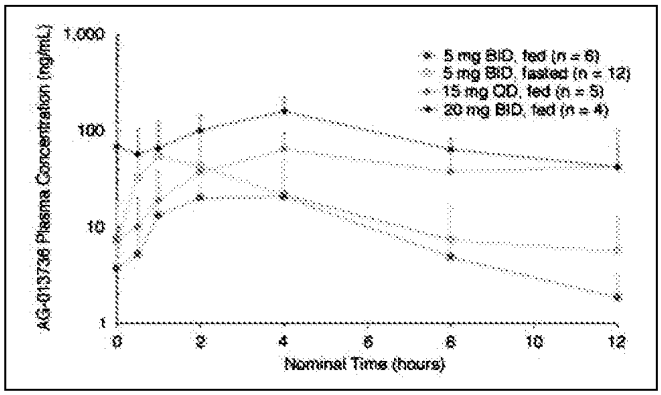

Fig 2. Area under the curve (AUC) and maximum plasma concentration (C_{max}) versus dose: linearity in pharmacokinetics. QD, every day.

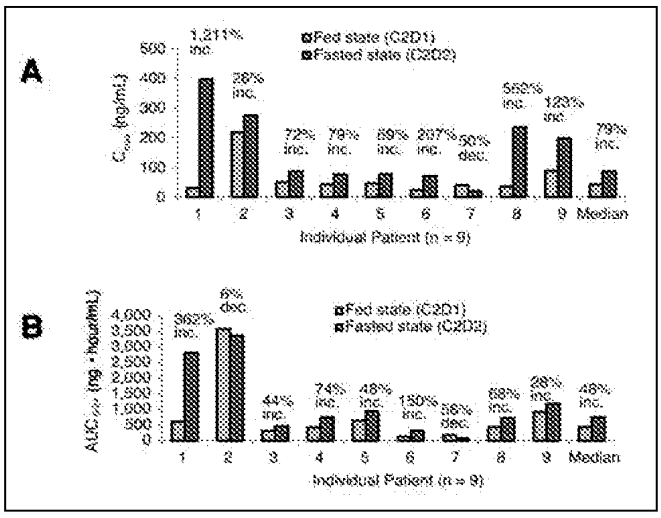

Fig 3. Effect of food on AG-013736 (A) maximum plasma concentration (C_{max}) and (B) area under the curve (AUC).

evaluated for response according to RECIST. There were three confirmed partial responses and other indications of antitumor activity. One patient with adenoid cystic carcinoma enrolled in the 15-mg-QD cohort was identified as a responder after completing three cycles. The response was ongoing 4 months later, when the patient stopped treatment because of subjectively intolerable grade 2 stomatitis accompanied by weight loss. Partial response was also observed in two of six patients with renal cell carcinoma (RCC). In one of these patients, response was identified after 10 weeks of treatment with 5 mg BID in the fasted state. Disease progression was documented 18 weeks later. The second patient with RCC was identified as a responder after two cycles. One

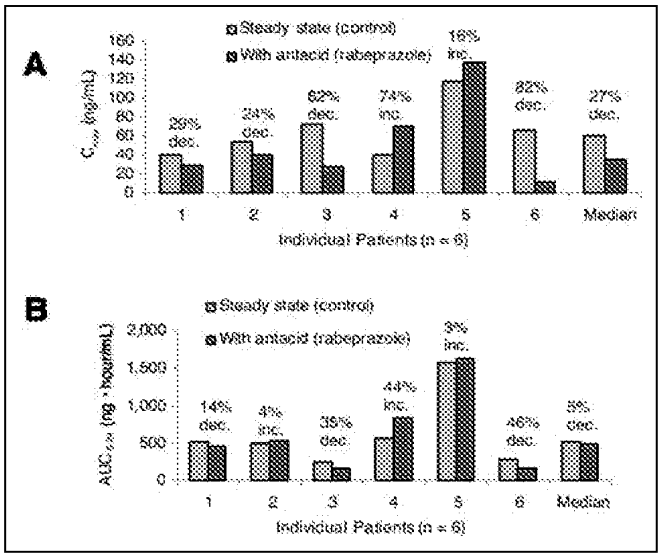

Fig 4. Effect of the proton-pump inhibitor rabeprazole on (A) maximum plasma concentration (C_{max}) and (B) area under the curve (AUC).

Table 4. Dose-Limiting Toxicities (DLTs)

Cohort (starting dose, No. of patients)	No. of Patients With DLTs	Dose-Limiting Toxicity (No. of patients)
1 (various* fed, 6)	3	Hypertension (3); increased liver function tests (2); seizure (2); apnea (1)
2 (20 mg BID fed, 4)	3	Hypertension (2); hemoptysis (1); stomatitis (1)
3 (5 mg BID fed, 6)	0	None
4 (15 mg QD fed, 6)	3	Hypertension (1); stomatitis (1); pancreatitis, ischemic bowel, and thromboembolism (1)
5 (5 mg BID fasted, 8)	1	Stomatitis (1)
6 (2 mg BID then 5 mg BID fasted, 6)	1	Diarrhea (1)

Abbreviations: BID, twice daily; QD, once daily.
*The following doses were administered: 10 mg QD (one patient), 10 mg BID (two patients), 20 mg BID (two patients), and 30 mg BID (two patients).

year later, the patient stopped treatment with AG-013736 to be evaluated for surgical excision of his remaining lung metastases. Four weeks later, his disease progressed such that surgery was no longer an option. AG-013736 treatment was restarted, resulting in renewed tumor shrinkage (Fig 5).

Cavitation of lung lesions was observed in the two patients with non–small-cell lung cancer (Fig 6). Although both patients subsequently died with hemoptysis (only one of the deaths was considered related to AG-013736), this may be evidence of a significant antiangiogenic effect of AG-013736. Three additional patients had decreases in tumor burden that did not qualify by RECIST criteria for response, including one patient each with mesothelioma (on treatment for 16 months), thyroid cancer (9 months), and RCC (4 months). Finally, a patient with refractory breast cancer experienced healing of skin lesions before starting treatment with the CYP3A inducer phenytoin (see Discussion for details).

Table 5. Summary of Toxicities by Severity (National Cancer Institute common terminology criteria grading system)

Adverse Event*	All Grades		Grades 3-4	
	No. of Patients	%	No. of Patients	%
Hypertension	22	61	11	30
Fatigue	10	28	—	—
Nausea	7	19	—	—
Diarrhea	6	17	1	3
Vomiting	5	14	—	—
Headache	5	14	—	—
Stomatitis	4	11	2	6
Erythema	4	11	—	—
Proteinuria	3	8	1	3
Elevated ALT	2	6	2	6
Elevated AST	2	6	2	6
Convulsions	2	6	2	6
Urinary frequency	1	3	1	3
Apnea	1	3	1	3
Oral candiasis	1	3	1	3
Hemoptysis	1	3	1	3
Hypercholesterolemia	1	3	1	3
Intestinal ischemia	1	3	1	3
Pancreatitis	1	3	1	3
Thromboembolism	1	3	1	3

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.
*Only laboratory abnormalities that resulted in dose reduction, treatment interruption, or clinical sequelae, or were otherwise considered important by the investigator were recorded as adverse events.

DISCUSSION

This phase I study has defined the MTD for AG-013736 and identified important issues regarding absorption, drug interactions, and toxicities. In addition, data from this trial support the clinical activity of AG-013736 in advanced and refractory cancer, providing the information necessary to proceed with phase II testing in specific malignancies.

As is commonly seen with orally administered drugs, PK was variable; the major determinant for variability is not clear at this time. In vitro studies with human liver indicate that AG-013736 is metabolized by conversion to glucuronide metabolites as well as oxidation by the CYP3A4 and CYP1A2 isozymes, two P450 enzymes known to be

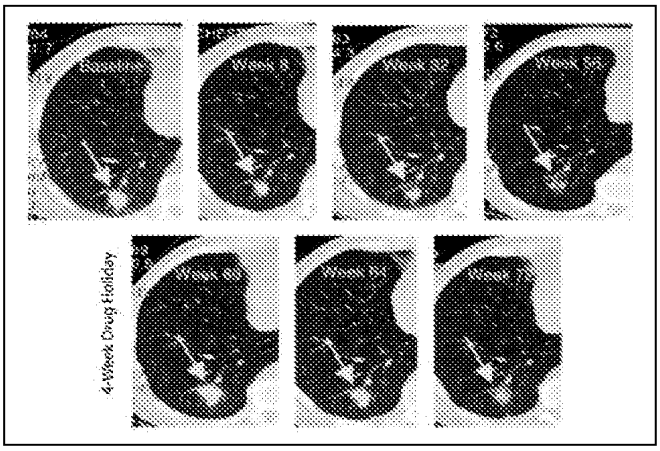

Fig 5. Partial remission in a patient with renal cell carcinoma with renewed tumor shrinkage after a drug holiday.

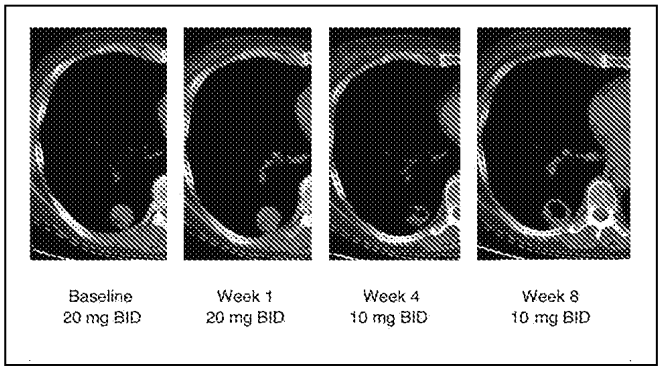

Fig 6. Cavitation of lung metastasis in a patient with adenocarcinoma of the lung.

inducible and thus likely to exhibit variability in patients. Additionally, the CYP3A4 isozyme content in human intestine and liver is known to be highly variable even in healthy (noninduced) individuals.

Another contributor toward the PK variability could be differences in AG-013736 gastrointestinal absorption. PK testing in this trial revealed that dosing in the fasted state resulted in the best absorption; this recommendation has been incorporated into phase II trials. It is interesting to note that although the aqueous solubility of AG-013736 is pH dependent with low (acidic) pH, resulting in the highest solubility, drug concentrations in this study were not significantly affected by the proton-pump inhibitor rabeprazole. Several clinical evaluations in healthy volunteers are ongoing or planned to better understand sources of variability in the oral PK of AG-013736.

Data from a single patient in cohort one indicated a likely drug interaction with phenytoin. After phenytoin use, the $AUC_{0\text{-}24}$ and C_{max} of AG-013736 were reduced approximately 10-fold. Phenytoin is a potent inducer of multiple CYP450 enzymes, and it is likely that it induced AG-013736 clearance. Although there had been clinical evidence of healing of malignant and ulcerating skin lesions in this patient before the start of phenytoin, she subsequently experienced disease progression that resulted in discontinuation from study treatment. Concomitant use of potent inducers of the CYP3A4 and CYP1A2 isozymes was subsequently excluded during treatment with AG-013736.

The initial doses used in this trial were clearly above the MTD for AG-013736. Nonclinical safety studies conducted to support the phase I starting dose did not identify the hypertensive effect of AG-013736, which resulted in the clinical administration of the maximum administrable dose within the first two cohorts. In addition, DLT was not identified in cohort one (partly because the need for daily blood pressure monitoring had not been recognized yet) before enrollment was permitted by protocol into cohort two. This led to the selection of the cohort-two dose (20 mg BID) based on the relatively unremarkable early safety profile of patients in cohort one, who were receiving doses between 10 mg QD and 30 mg BID. The subsequent dose de-escalation and study amendment to monitor and treat hypertension adequately avoided additional significant toxicity. The experiences described in this trial highlight the importance of well-designed phase I studies with detailed PK sampling to better understand human-specific end-organ toxicities, potential drug interactions affecting therapeutic blood concentrations, and therapeutic dosing.

The observed toxicities are similar to those observed for other antiangiogenic agents,[26-30] with some variability in DLT, and are dose related. Diarrhea and nonulcerating stomatitis (glossodynia) were generally tolerable. Hypertension, which was easily controlled with antihypertensive medications in patients receiving the recommended phase II dose of AG-013736, has also been seen with the antibody to VEGF, bevacizumab,[31,32] as well as with oral angiogenesis inhibitors.[27,33] The development of hypertension is likely to be related to a mechanistic effect of antiangiogenic agents and may be a marker of VEGFR inhibition. However, increases in indices of vascular stiffness seen with the angiogenesis inhibitor BAY 43-9006 do not correlate with levels of circulating VEGF.[33] In addition to its role in triggering angiogenesis, VEGF exerts a stimulatory effect on endothelial nitric oxide (NO) production by upregulating endothelial nitric oxide synthase expression. NO in turn has a vasodilatory effect.[34-36] Inhibition of VEGFR signaling by AG-013736 may cause a decrease in NO production, leading to vasoconstriction and hypertension in a subset of patients. Preclinical data support this hypothesis; in vitro evaluation of AG-013736 in human umbilical vein endothelial cells revealed inhibition of endothelial nitric oxide synthase activity at the median infectious dose that inhibited VEGFR-2 phosphorylation.[37]

AG-013736 has clear clinical activity in advanced and refractory malignancies, as shown in this phase I trial. Sustained tumor response was seen in renal cell cancer as well as in adenoid cystic cancer, and antitumor activity was demonstrated in patients with lung cancer. Tumor necrosis with hemoptysis in centrally located lung lesions has been seen also with bevacizumab and may be a specific marker of antiangiogenic activity. Earlier treatment of lung carcinomas and careful selection of patients could avoid this potentially life-threatening toxicity. The evaluation of pharmacodynamic response to AG-013736 measured by DCE magnetic resonance imaging, reported separately, demonstrated a decrease in tumor vascular parameters at the selected phase II dose.

Based on the encouraging clinical results from this trial, evidence of antiangiogenic activity on DCE magnetic resonance imaging, and the tolerability of AG-013736 at a dose of 5 mg BID in the fasted state, current and planned phase II trials are evaluating efficacy as a single agent or in combination with chemotherapy in a variety of malignancies. A variety of anti-VEGFR small molecules, differentiated by target and

specificity, are in development.[26,30,38-40] The multitargeted nature of these molecules, for example combining VEGF and PDGFR inhibition, may be critical to antitumor effects[19,41] and may be a specific advantage over antibodies that target the ligand alone. As we continue to explore the use of potent antiangiogenic agents in the treatment of cancer, it will be important to include appropriate PK evaluations, as well as surrogate markers to evaluate target inhibition.[22,42-45]

Acknowledgment

We gratefully acknowledge the help of Marina Kenzer, Tracey Borst, Donna Alberti, Kimberly Binger, Cathy Henceroth, Kavitha Krishnan, Michelle Purdom, Debra Chicks, and Krista McAlee for patient recruitment, data management, and patient care during the conduct of this phase I trial. We also thank Brian Hee for assistance with bioanalysis of the pharmacokinetics samples. Finally, we appreciate the efforts of Anton Mulvaney, who was responsible for study management.

Authors' Disclosures of Potential Conflicts of Interest

Although all authors have completed the disclosure declaration, the following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Authors	Employment	Leadership	Consultant	Stock	Honoraria	Research Funds	Testimony	Other
Hope S. Rugo					Pfizer (A)	Pfizer (A)		
Roy S. Herbst			Pfizer (A)			Pfizer (C)		
Glenn Liu			Pfizer (A)					
John W Park			Pfizer (A)		Pfizer (A)	Pfizer (C)		
Heidi M. Steinfeldt	Pfizer							
Yazdi K. Pithavala	Pfizer			Pfizer (C)				
Steven D. Reich	Pfizer							
James L. Freddo	Pfizer			Pfizer (B)				
Dollar Amount Codes (A) < $10,000 (B) $10,000-99,999 (C) ≥ $100,000 (N/R) Not Required								

References

1. Folkman J: Fundamental concepts of the angiogenic process. Curr Mol Med 3:643-651, 2003
2. Hanahan D, Folkman J: Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86:353-364, 1996
3. Folkman J: Role of angiogenesis in tumor growth and metastasis. Semin Oncol 29:15-18, 2002
4. Folkman J, Watson K, Ingber D, et al: Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 339:58-61, 1989
5. Huss WJ, Hanrahan CF, Barrios RJ, et al: Angiogenesis and prostate cancer: Identification of a molecular progression switch. Cancer Res 61:2736-2743, 2001
6. Gasparini G, Harris AL: Clinical importance of the determination of tumor angiogenesis in breast carcinoma: Much more than a new prognostic tool. J Clin Oncol 13:765-782, 1995
7. Hayes AJ, Li LY, Lippman ME: Science, medicine, and the future. Antivascular therapy: A new approach to cancer treatment. BMJ 318: 853-856, 1999
8. Folkman J, D'Amore PA: Blood vessel formation: What is its molecular basis? Cell 87:1153-1155, 1996
9. Senger DR: Molecular framework for angiogenesis: A complex web of interactions between extravasated plasma proteins and endothelial cell proteins induced by angiogenic cytokines. Am J Pathol 149:1-7, 1996
10. Shinkaruk S, Bayle M, Lain G, et al: Vascular endothelial cell growth factor (VEGF), an emerging target for cancer chemotherapy. Curr Med Chem Anti-Canc Agents 3:95-117, 2003
11. Ferrara N: Vascular endothelial growth factor: Basic science and clinical progress. Endocr Rev 25:581-611, 2004
12. Fontanini G, Vignati S, Boldrini L, et al: Vascular endothelial growth factor is associated with neovascularization and influences progression of non-small cell lung carcinoma. Clin Cancer Res 3:861-865, 1997
13. Takahashi R, Tanaka S, Kitadai Y, et al: Expression of vascular endothelial growth factor and angiogenesis in gastrointestinal stromal tumor of the stomach. Oncology 64:266-274, 2003
14. Bellamy WT, Richter L, Frutiger Y, et al: Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies. Cancer Res 59:728-733, 1999
15. Hlatky L, Hahnfeldt P, Folkman J: Clinical application of antiangiogenic therapy: Microvessel density, what it does and doesn't tell us. J Natl Cancer Inst 94:883-893, 2002
16. Poon RT, Ng IO, Lau C, et al: Tumor microvessel density as a predictor of recurrence after resection of hepatocellular carcinoma: A prospective study. J Clin Oncol 20:1775-1785, 2002
17. Folkman J: Angiogenesis inhibitors: A new class of drugs. Cancer Biol Ther 2:S127–S133, 2003
18. Glade-Bender J, Kandel JJ, Yamashiro DJ: VEGF blocking therapy in the treatment of cancer. Expert Opin Biol Ther 3:263-276, 2003
19. Bergers G, Song S, Meyer-Morse N, et al: Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest 111:1287-1295, 2003
20. Vulfovich M, Saba N: Molecular biological design of novel antineoplastic therapies. Expert Opin Investig Drugs 13:577-607, 2004
21. Inai T, Mancuso M, Hashizume H, et al: Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts. Am J Pathol 165:35-52, 2004
22. Liu G, Rugo HS, Wilding G, et al: Dynamic contrast-enhanced magnetic resonance imaging as a pharmacodynamic measure of response after acute dosing of AG-013736, an oral angiogenesis inhibitor, in patients with advanced solid tumors: Results from a phase I study. J Clin Oncol (in press)
23. DeGeorge J, Ahn C-H, Andres P, et al: Regulatory considerations for preclinical development of anticancer drugs. Cancer Chemother Pharmacol 41:173-185, 1998
24. Cancer Therapy Evaluation Program: Common Terminology Criteria, Version 2.0. Division of Cancer Treatment & Diagnosis, National Cancer Institute, National Institutes of Health, 1998, Department of Health and Human Services, April 30, 1999

25. Therasse P, Arbuck SG, Eisenhower EA, et al: New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92:205-216, 2000

26. Rosen L, Mulay M, Long J, et al: Phase I trial of SU011248, a novel tyrosine kinase inhibitor in advanced solid tumors. Proc Am Soc Clin Oncol 22:191, 2003 (abstr 765)

27. Stopeck A, Sheldon M, Vahedian M, et al: Results of a Phase I dose-escalating study of the antiangiogenic agent, SU5416, in patients with advanced malignancies. Clin Cancer Res 8:2798-2805, 2002

28. Thomas AL, Morgan B, Drevs J, et al: Vascular endothelial growth factor receptor tyrosine kinase inhibitors: PTK787/ZK 222584. Semin Oncol 30:32-38, 2003

29. O'Farrell AM, Foran JM, Fiedler W, et al: An innovative phase I clinical study demonstrates inhibition of FLT3 phosphorylation by SU11248 in acute myeloid leukemia patients. Clin Cancer Res 9:5465-5476, 2003

30. George D, Michaelson D, Oh WK, et al: Phase I study of PTK787/ZK 222584 (PTK/ZK) in metastatic renal cell carcinoma. Proc Am Soc Clin Oncol 22:385, 2003 (abstr 1548)

31. Hurwitz H, Fehrenbacher L, Novotny W, et al: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335-2342, 2004

32. Rugo HS: Bevacizumab in the treatment of breast cancer: Rationale and current data. Oncologist 9:43-49, 2004 (suppl 1)

33. Veronese ML, Flaherty R, Townsend A, et al: Pharmacodynamic study of the raf kinase inhibitor BAY 43-9006: Mechanisms of hypertension. Proc Am Soc Clin Oncol 22:135s, 2004 (abstr 2035)

34. Bouloumie A, Schini-Kerth VB, Busse R: Vascular endothelial growth factor up-regulates nitric oxide synthase expression in endothelial cells. Cardiovasc Res 41:773-780, 1999

35. Kroll J, Waltenberger J: A novel function of VEGF receptor-2 (KDR): rapid release of nitric oxide in response to VEGF-A stimulation in endothelial cells. Biochem Biophys Res Commun 265:636-639, 1999

36. Shiojima I, Walsh K: Role of Akt signaling in vascular homeostasis and angiogenesis. Circ Res 90:1243-1250, 2002

37. Wickman G, Hallin M, Salansky KA, et al: Further characterization of the potent VEGF/PDGF receptor tyrosine kinase inhibitor AG-013736 in preclinical tumor models for its antiangiogenesis and antitumor activity. Proc Am Assoc Cancer Res 44:865, 2003 (abstr 3780)

38. Sun L, McMahon G: Inhibition of tumor angiogenesis by synthetic receptor tyrosine kinase inhibitors. Drug Discov Today 5:344-353, 2000

39. Kerbel R, Folkman J: Clinical translation of angiogenesis inhibitors. Nat Rev Cancer 2:727-739, 2002

40. Ciardiello F, Bianco R, Caputo R, et al: Antitumor activity of ZD6474, a vascular endothelial growth factor receptor tyrosine kinase inhibitor, in human cancer cells with acquired resistance to antiepidermal growth factor receptor therapy. Clin Cancer Res 10:784-793, 2004

41. Bergers G, Javaherian K, Lo KM, et al: Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. Science 284:808-812, 1999

42. Mundhenke C, Thomas JP, Wilding G, et al: Tissue examination to monitor antiangiogenic therapy: A phase I clinical trial with endostatin. Clin Cancer Res 7:3366-3374, 2001

43. O'Farrell AM, Yuen HA, Smolich B, et al: Effects of SU5416, a small molecule tyrosine kinase receptor inhibitor, on FLT3 expression and phosphorylation in patients with refractory acute myeloid leukemia. Leuk Res 28:679-689, 2004

44. Willett CG, Boucher Y, di Tomaso E, et al: Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 10:145-147, 2004

45. Tong RT, Boucher Y, Kozin SV, et al: Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res 64:3731-6, 2004